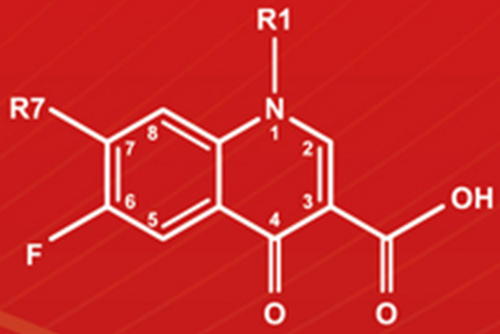
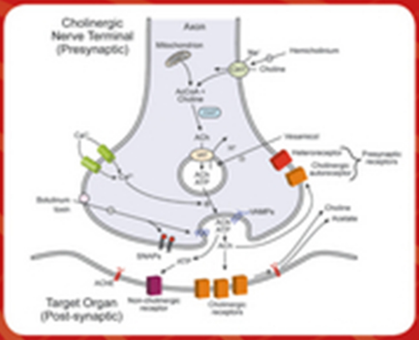
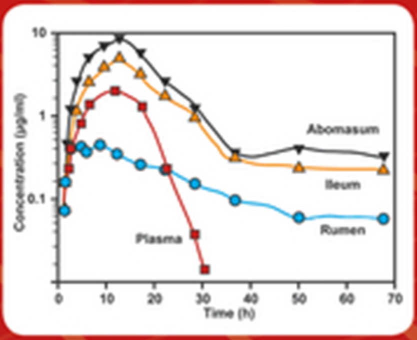
Tenth Edition

Veterinary Pharmacology & Therapeutics

Edited by
Jim E. Riviere
Mark G. Papich







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WILEY Blackwell

Veterinary Pharmacology and Therapeutics

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Tenth Edition

Edited by

Jim E. Riviere, DVM, PhD, DSc (hon) and Mark G. Papich, DVM, MS

WILEY Blackwell

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In loving memory of my son Dr. Brian A. Riviere.

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Preface

This current volume marks the tenth edition of *Veterinary Pharmacology and Therapeutics*, first published some six decades ago by Dr. L. Meyer Jones. Both the discipline and book have dramatically changed since the first edition came out in 1954. Entirely new therapeutic classes of drugs are now used and veterinary pharmacotherapy is much more quantitative, mechanism-based, and firmly integrated into clinical medicine than in past years. Early editions were intended to be simply a standard textbook for students taking a pharmacology course in the veterinary curriculum. However, the educational culture and environment of veterinary colleges have evolved, making the audience of the current edition much broader than the veterinary medical student alone. Interns and residents in various medical disciplines, including clinical pharmacology, graduate students in comparative biomedical sciences, laboratory animal specialists, animal health drug developers and regulators, and as always researchers across diverse fields, all view this text as the standard reference source and supplemental reading for comparative pharmacology at all levels of professional and graduate school curriculums. This role as the primary veterinary pharmacology reference textbook has become even more important as so-called open textbook initiatives become mandatory in more veterinary colleges.

Because of the unique role this text serves in comparative and veterinary pharmacology across the globe, we have endeavored to significantly update and revise this current edition with substantial rewriting and editing of the majority of chapters. We have enlisted the collaboration of authors who are experts in their respective fields and instructed them to focus on drugs that are currently used in veterinary practice. To accommodate these additions, we have removed discussion of older drugs no longer routinely employed and instead direct the reader interested in these compounds to consult earlier editions of the text. As in the last edition, we have also presented therapeutic agents both by pharmacological class but also regrouped into therapeutic applications to insure clinical relevance. We remain cognizant of the public health responsibilities of veterinarians in production animal practice and have endeavored to discuss issues related to updated regulations, as well as avoidance of both antimicrobial drug resistance and violative tissue residues in edible products from food-producing animals.

We are indebted to the authors of the text who tried as best as possible to adhere to deadlines despite their busy schedules, to the excellent support of the publisher's staff at Wiley-Blackwell, as well as the numerous staff and students at both Kansas State and North Carolina State Universities who aided in arriving at this final product. We hope the reader finds that this tenth edition of *Veterinary Pharmacology and Therapeutics* serves them well and provides a solid foundation for the rational use of drugs in veterinary medicine.

Jim E. Riviere and Mark G. Papich

About the Companion Website

This book is accompanied by a companion website:

www.wiley.com/go/riviere/pharmacology

The website includes:

- Figures from the book in PowerPoint

Section 1

Principles of Pharmacology

1

Veterinary Pharmacology: An Introduction to the Discipline

Jim E. Riviere and Mark G. Papich

Pharmacology is the science that broadly deals with the physical and chemical properties, actions, absorption, and fate of chemical substances termed *drugs* that modify biological function. It is a discipline that touches most areas of human and veterinary medicine and closely interfaces with pharmaceutical science and toxicology.

History of Pharmacology

As long as humans and their animals have suffered from disease, chemical substances have played a role in their treatment. Substances obtained from plants and animals or their products were used according to precise prescriptions through antiquity. The mechanism attributed to why these substances were effective are deeply rooted in the beliefs and mythologies of each culture, as were the rituals involved in their preparation.

The early history of pharmacology parallels human efforts to compile records of ailments and their remedies. The earliest recorded compilation of drugs, the *Pen Tsao*, consisted of a list of herbal remedies compiled in the reign of Chinese Emperor Shennung in 2700 B.C. Classic examples of medicinal use of chemicals, herbs, and other natural substances are found in the recorded papyri of ancient Egypt. The *Kahun Papyrus,* written about 2000 B.C., lists prescriptions for treating uterine disease in women and specifically addresses veterinary medical concerns. The *Ebers Papyrus,* written in 1150 B.C. is a collection of folklore covering 15 centuries of history. It is composed of over 800 prescriptions for salves, plasters, pills, suppositories, and other dosage forms used to treat specific ailments.

The ancient Greek philosopher–physicians of 500 B.C. taught that health was maintained by a balance of "humors," which were affected by temperature, humidity, acidity, and sweetness, rather than to the direct actions of gods or demons. Disease was treated by returning these humors to a proper balance. Hippocrates (460–370 B.C.) was an ancient Greek physician of the Age of Pericles. He is referred to as the "father of medicine" in recognition of his lasting contributions to the field as the founder of the Hippocratic school of medicine. He was a firm believer in the healing powers of nature, conducted systematic observations of his patients' symptoms, and began moving the practice of medicine from an art to a systematic clinical science. The first true *material medica*, a compilation of therapeutic substances and their uses, was compiled in 77 A.D. by Aristotle's student Dioscorides, while serving as a surgeon in Nero's Roman Legion traveling throughout the Mediterranean. This served as the basis for the later works of Galen (131–201) that emerged as the authoritative material medica for the next 1,400 years! In fact, some pharmaceutical preparations consisting of primarily herbal or vegetable matter are still referred to as galenical preparations. As the Dark Ages descended upon Europe, such scholarship transferred to Byzantium, where in fact a veterinary compilation for farm animal treatments, *Publius Vegetius,* was compiled in the fifth century.

It took until the Renaissance to awaken the spirit of discovery in Europe. The Swiss physician Theophrastus Bombastus von Hohenheim (1492–1541), known as Paracelsus, introduced the clinical use of laudanum (opium) and a number of tinctures (extracts) of various plants, some of which are still in use today. He is remembered for using drugs for specific and directed purposes, and for the famous dictum "All substances are poisons; there is none which is not a poison. The proper dose separates a poison from a remedy." As these practices took root, official compilations of medicinal substances, their preparation, use, and dosages, started to appear in Europe. These publications, termed *pharmacopeia*, provided a unifying framework upon which the pharmaceutical sciences emerged. The first printed pharmacopeia, titled the *Dispensatorium,* was published by Valerius Cordus in 1547 in Nuremberg, Germany. Local publications emerged in different European cities, with two pharmacopeias published in London in 1618. The *Edinburgh Pharmacopoeia* published in 1689 became the most influential during this period. It took until the mid-19th century before truly national pharmacopeias took

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hold, with the first *United States Pharmacopeia* published in 1820. The first *United States Pharmacopeia* has been given the title USP-0; the current edition of the *United States Pharmacopeia* is titled USP-39 and the National Formulary titled NF 34, which includes excipients. There was also a British pharmacopeia first published in 1864 and the *British Pharmacopeia* continues to be published today.

The history of pharmacology parallels the development of modern medicine and the realization that specific natural products and substances may cure specific diseases. The 16th and 17th centuries were marked by great explorations and the beginning of medical experimentation. In 1656, Sir Christopher Wren made the first intravenous injection of opium in a dog. The bark of the cinchona tree was brought by Jesuits from South America for use of treatment of malaria. In 1783, the English physician William Withering reported on his experience in the use of extracts from the foxglove plant to treat patients with "dropsy," a form of edema most likely caused by congestive heart failure.

In the early 1800s the French physiologist–pharmacologist Megendie, working with the pharmacist Pelletier, studied the effects of intravenous injections of ipecac, morphine, strychnine, and other substances on animals. Megendie was the first to prove that chemicals can be absorbed into the vascular system to exert a systemic effect. A prolific scientist, he also published a formulary that survived through eight editions from 1821 to 1836. The Spanish physician Orfila published the results of many experiments in a book entitled *Toxicologie Generale* in 1813. A student of Megendie, the famous physiologist Claude Bernard, and others, showed in the mid-1800s that the active ingredient of foxglove botanical preparations was digitalis, and its action was on the heart. We continue to use digoxin today for the treatment of congestive heart failure in humans and animals. The important aspect of these early studies was that they used the experimental paradigm for demonstrating chemical activity, establishing both the philosophy and methods upon which the discipline of modern pharmacology is based.

The term *Pharmakologie* was applied to the study of material medica by Dale in London as early as 1692; however, it is generally regarded that the biochemist Rudolph Buchheim in the Baltic city of Dorpat established the first true experimental laboratory dedicated to pharmacology in the mid-18th century. He published some 118 contributions on a variety of drugs and their actions, and argued for pharmacology to be a separate discipline distinct from material medica, pharmacy, and chemistry. His work included in 1849 a textbook *Beiträge zur Ärzneimittellehre,* which classified drugs based on their pharmacological action in living tissue. He deleted traditional remedies if he could not demonstrate their action in his laboratory. This is the beginning of what we now know as *evidence-based pharmacology,* which requires that a chemical be termed a drug only if a specific action in living tissues can be demonstrated.

His student, Oswald Schmiedeberg, became a Professor of Pharmacology at the University of Strasbourg in 1872 and took upon himself the goal of making pharmacology an independent scientific discipline based upon precise experimental methodology that ultimately displaced material medica in medical school curriculums throughout Europe by the end of the 19th century and by the early 20th century in America. He studied the correlation between the chemical structure of substances and their effectiveness as narcotics. He published some 200 publications as well as an authoritative textbook in 1883 that went through seven editions. This text classified drugs by their actions and separated experimental pharmacology from therapeutics. In addition he founded and edited the first pharmacology journal *Archiv für experimentelle Pathologie und Pharmakologie* in 1875, which in 2007 published volume 375 as *Naunym-Schmiedeberg's Archives of Pharmacology.* His more than 150 students spread the discipline of pharmacology throughout Europe and America.

One of his students, Dr. John Abel, held the first full-time professorship in pharmacology at the University of Michigan and is considered by some to be the father of American pharmacology. Professor Abel then moved to Johns Hopkins Medical School where he continued his basic pharmacological research and founded the *Journal of Biological Chemistry* as well as the *Journal of Pharmacology and Experimental Therapeutics.* He was instrumental in founding the *American Society of Pharmacology and Experimental Therapeutics* in 1908.

From these origins, the various disciplines of pharmacology grew, the common factor being the focus in experimental methods to discover and confirm drug actions. Today, the basic philosophy remains unchanged, although modern techniques are grounded in analytical chemistry, mathematical models, and the emerging science of pharmacogenomics. Such so-called "big data" approaches coupled with high throughput screening approaches are the primary tools now used in drug discovery.

Veterinary Pharmacology

The development of veterinary pharmacology generally paralleled that of human pharmacology. However, there is archeological evidence of an Indian military hospital for horses and elephants from 5000 B.C., at which time there also existed an extensive medical education program at the Hindu university at Takkasila. The formal discipline of veterinary pharmacology has its origins in the establishment of veterinary colleges and hospitals in France, Austria, Germany, and the Netherlands in the 1760s as a response to epidemics of diseases such as rinderpest that decimated animal populations throughout Western Europe. The Royal College of Veterinary Surgeons was established in London in 1791 followed in 1823 by the Royal (Dick) School of Veterinary Studies in Edinburgh. The earliest veterinary colleges were established in the United States in 1852 in Philadelphia and in Boston in 1854; however, both were short-lived. Modern existing North American veterinary schools founded in the late 1800s, and which continue in operation, include those in Iowa, Ohio, Ontario, Pennsylvania, and New York.

In these early colleges, teaching of pharmacology in veterinary schools was essentially *materia medica*, and remained closely aligned with parallel efforts occurring in medical schools, especially when colleges were located on the same campuses. This was evident in the European schools, with a separation really occurring in the 20th century. However, this linkage was not absolute. An early mid-19th century veterinary textbook *The Veterinarian's Vade Mecum* was published by John Gamgee in England. It was essentially a *materia medica* and did not reflect the biological-based classification system for substances used by Professor Buchheim in the same period. The first American professor of therapeutics at the School of Veterinary Medicine at Iowa State was a physician, D. Fairchild. Similarly, a textbook of veterinary pharmacology *Veterinary Material Medica and Therapeutics* published by the School of Veterinary Medicine at Harvard was authored by Kenelm Winslow, a veterinarian and physician. This book, the eighth edition of which was published in 1919, began to follow the modern thrust described earlier of relating drug actions to biological effects on tissues. It seems veterinary medicine's 21st-century preoccupation with the "one-medicine" concept has deep historical roots.

The history of veterinary pharmacology has been reviewed by Lees, Fink-Gremmels, and Toutain (2013). They reviewed the past history and current status of the science, particularly the developments in the last 35 years. The authors analyzed publication data and showed the tremendous increase in scientific articles focusing on veterinary pharmacology since 1975. The important event, which fully shifted veterinary pharmacology from one focused on *materia medica* to the actual science of pharmacology, was the publication by Professor L. Meyer Jones in 1954 of the first edition of the textbook you are now reading. From this point forward, veterinary pharmacology positions have existed in Colleges of Veterinary Medicine throughout the world, the structure of which are often a reflection of local university history, priorities, and academic structure.

Organized veterinary pharmacology occurred rather simultaneously in Europe and the Americas. The American Academy of Veterinary Pharmacology and Therapeutics (AAVPT) was founded in 1977 and the European Association for Veterinary Pharmacology and Toxicology (EAVPT) in 1978. These two organizations, together with the British Association for Veterinary Clinical Pharmacology and Therapeutics, launched the *Journal of Veterinary Pharmacology and Therapeutics* (JVPT) in 1978. Its founder, Dr. Andrew Yoxall, hoped that the journal would improve coordination and communication among pharmacologists and veterinary clinicians, and designed it for the publication of topics relating both to the clinical aspects of veterinary pharmacology, and to the fundamental pharmacological topics of veterinary relevance. Now in its 40th year of publication, edited by Dr. Riviere and also cosponsored by both the American College of Veterinary Clinical Pharmacology (ACVCP) and the Chapter of Veterinary Pharmacology of the Australian College of Veterinary Scientists, this journal remains the primary outlet for publication of veterinary-related science-based pharmacology investigations.

The discipline of clinical pharmacology is more directly related to applying pharmacological principles – particularly pharmacokinetics – to clinical patients. Fellows of the AAVPT formed the AVMA-recognized board certified specialty – the American College of Veterinary Clinical Pharmacology (ACVCP) – in 1991. The establishment of the ACVCP paralleled the establishment of the American Board of Clinical Pharmacology (ABCP) – the human medical counterpart – in the same year with the cooperation of the American College of Clinical Pharmacology. In order to promote a focus on applying higher-level mathematical techniques to veterinary pharmacology, the international Animal Health Modeling and Simulation Society (AHM&S) was founded in 2012.

Regulations and Standards

A different perspective on the development of veterinary pharmacology over the last century is the development of regulatory bodies to insure that safe, effective, and pure drugs reach commerce. As discussed above, *material medica* and *pharmacopeia* were in large part the force that held pharmacology together as a discipline for centuries. Since 1820, the *United States Pharmacopeia (USP)*, a private, not-for-profit organization, has endeavored to establish standards for strength, quality, purity, packaging, and labeling for all manufacturers of pharmaceutical substances in the United States. It took until 1990, under the pressure of Dr. Lloyd Davis, one of the founding fathers of AAVPT and ACVCP, to have USP specifically develop Committees to develop USP standards and information for veterinary drugs. Until this time, veterinary drugs whose manufacturers desired the "USP Label" processed drugs through committees that were largely populated by experts in human pharmaceutical sciences and medicine. Additional contributions of veterinary pharmacists to our discipline are described in Chapter 56 of this book.

In the 20th century, due to the proliferation of charlatans and fraud in manufacture and distribution of so-called "pure" medicinal products, coupled with serious human health calamities due to nonregulated drugs reaching the market, Congress in 1927 established the Food, Drug and Insecticide Administration, which later became known as the Food and Drug Administration (FDA). In 1938, the pivotal Federal Food, Drug and Cosmetic Act was passed, giving the FDA the authority to regulate animal drugs by requiring evidence of product safety before distribution. In 1959, a veterinary medical branch was developed as a division and the Food Additive Amendments Act was passed, which gave FDA authority over animal food additives and drug residues in animal-derived foods. A Bureau of Veterinary Medicine, with Dr. M. Clarkson as its first director, was established in 1965 to handle the increasing regulatory responsibilities of animal drugs. Today, the FDA Center for Veterinary Medicine, directed by Dr. Steven Solomon, is the primary regulatory body for veterinary drugs in the United States. The interested reader should consult Chapter 55 of the present text for a more in-depth discussion of the current state of veterinary regulatory authority.

In addition to the standard-setting activities of the USP, veterinary pharmacologists have contributed to other standard-setting organizations. Veterinary pharmacology has had its influence on national and international standard-setting organizations and is contributing to the "one health" concept of medicine. Veterinary pharmacologists and microbiologists have worked together to form the Veterinary Susceptibility Testing Subcommittee (VAST) of the Clinical and Laboratory Standards Institute (CLSI, formerly called NCCLS, www.CLSI.org). This subcommittee first met in 1993 and has now published four editions and three supplements of the public standards for identifying, and testing antimicrobial susceptibility for bacteria isolated from animals. The CLSI-VAST is still the only standard for testing antimicrobial susceptibility for animal pathogens in the world and contributes to the global effort to monitor antimicrobial resistance among bacteria.

What is Veterinary Pharmacology?

As can be appreciated from the breadth of material covered by the present textbook, veterinary pharmacology covers all aspects of using chemical and biological substances to treat diseases of animals. The basic principles of drug action are identical across veterinary and human pharmacology. Thus the principles of absorption, distribution, metabolism, and elimination covered here are the same as in any human pharmacology text, except for a focus on crucial species differences in anatomy, physiology, or metabolism that would alter these processes. The topics of pharmacodynamics, pharmacogenomics, and pharmacokinetics are also species-independent in basic concepts. These topics encompass what truly should be termed *comparative pharmacology*.

The subspecialties of veterinary pharmacology cover all those seen in human pharmacology, the classification of which can be seen from the division of the present text. These include classifying drugs as acting on the nervous, inflammatory, cardiovascular, renal, endocrine, reproductive, ocular, gastrointestinal, respiratory, and dermal systems as well as those used in chemotherapy of microbial, parasitic, and neoplastic diseases. Because of the potential exposure and heavy parasite load of both companion and production animals, antiparasitic drugs will get deeper coverage than seen in a human pharmacology text. There are a number of specialty areas that also reflect unique aspects of veterinary medicine, including aquatic, zoo, and avian species, as well as aspects of regulations related to racing animals and using drugs in food-producing animals with the resulting production of chemical residues and potential human food safety issues. This is simply not an issue in human medicine.

The discipline is often simply divided into basic and clinical pharmacology, the distinction being whether studies are conducted in healthy or diseased animals, studying experimental models or natural disease states, or involve laboratory or clinical studies in an actual veterinary clinical situation. However, the common denominator that separates a veterinary pharmacologists from our human pharmacology colleagues is dealing with species differences in both disposition and action of drugs.

Comparative pharmacology is the true common theme that courses through the blood of all veterinary pharmacologists, be they basic or clinical in orientation. How does a drug behave in the species being treated? Is the disease pathophysiology similar across species? Do dosages need to be adjusted? Are microbial susceptibilities for pathogens different? Is a drug absorbed, eliminated, or metabolized differently in a particular species or breed? Can the dosage form developed for a dog be used in an equine patient? Are there unique individual variations in the population due to pharmacogenomic variability that would alter a drug's effect in this patient? Are there unique species-specific toxicological effects for the drug in this patient? Is there a potential for drug–drug, drug–diet, or drug–environment interactions? Will this animal or its products be consumed by humans as food, and thus are potential residues from drug therapy a concern? All of these questions are addressed in the chapters that follow in this textbook.

The focus of veterinary pharmacology is to provide a rational basis for the use of drugs in a clinical setting in different animal species. These principles are fully discussed in the remainder of this text. The practicing veterinarian should appreciate every day that when a drug is given to a patient in his/her care, an experiment in clinical pharmacology is being conducted. The astute and successful practitioner will use principles of pharmacology to assure that the correct drug and dosage regimen is selected for the diagnosis in hand, that proper clinical outcomes will be assessed for both assuring efficacy and avoiding adverse effects, and finally that if a food-producing animal is being treated, proper caution is taken to insure the safety of animal-derived products to the human consumer.

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2

Absorption, Distribution, Metabolism, and Elimination

Jim E. Riviere

The four key physiological processes that govern the time course of drug fate in the body are absorption, distribution, metabolism, and elimination, the so-called *ADME processes*. Pharmacokinetics, the study of the time course of drug concentrations in the body, provides a means of quantitating ADME parameters. When applied to a clinical situation, pharmacokinetics provides the practitioner with a useful tool to design optimally beneficial drug dosage schedules for each individual patient. In the research and premarketing phase of drug development, it is an essential component in establishing effective yet safe dosage forms and regimens. An understanding of pharmacokinetic principles allows more rational therapeutic decisions to be made. In food animals, pharmacokinetics provides the conceptual underpinnings for understanding and utilizing the withdrawal time to prevent violative drug residues from persisting in the edible tissues of food-producing animals. A working knowledge of this discipline provides the framework upon which many aspects of pharmacology can be integrated into a rational plan for drug usage.

An Overview of Drug Disposition

To fully appreciate the ADME processes governing the fate of drugs in animals, the various steps involved must be defined and ultimately quantitated. The processes relevant to a discussion of the absorption and disposition of a drug administered by the intravenous (IV), intramuscular (IM), subcutaneous (SC), oral (PO), or topical (TOP) routes are illustrated in Figure 2.1. The normal reference point for pharmacokinetic discussion and analysis is the concentration of free, non-protein-bound drug dissolved in the serum (or plasma), because this is the body fluid that carries the drug throughout the body and from which samples for drug analysis can be readily and repeatedly collected. For the majority of drugs studied, concentrations in the systemic circulation are in equilibrium with the extracellular fluid of well-perfused tissues; thus, serum or plasma drug concentrations generally reflect extracellular fluid drug concentrations.

A fundamental axiom of using pharmacokinetics to predict drug effect is that the *drug must be present at its site of action in a tissue at a sufficient concentration for a specific period of time to produce a pharmacological effect.* Since tissue concentrations of drugs are reflected by extracellular fluid and thus serum drug concentrations, a pharmacokinetic analysis of the disposition of drug in the scheme outlined in Figure 2.1 is useful to assess the activity of a drug in the in vivo setting.

This conceptualization is especially important in veterinary medicine where species differences in any of the ADME processes may significantly affect the extent and/or time course of drug absorption and disposition in the body. By dividing the overall process of drug fate into specific phases, this relatively complex situation can be more easily handled. It is the purpose of this chapter to overview the physiological basis of absorption, distribution, metabolism, (biotransformation) and elimination. This will provide a basis for the chapter on pharmacokinetics that will deal with quantitating these processes in more detail.

Despite the myriad of anatomical and physiological differences among animals, the biology of drug absorption and distribution, and in some cases even elimination, is very similar in that it involves drug molecules crossing a series of biological membranes. As illustrated in Figure 2.2, these membranes may be associated with either several layers of cells (tissue) or a single cell, and both living and dead protoplasm may be involved. Despite the different biochemical and morphological attributes of each of these membranes, a unifying concept of biology is the basic similarity of all membranes, whether they be tissue, cell, or organelle. Although the specific biochemical components may vary, the fundamental organization is the same. This fact simplifies the understanding of the major determinants of drug absorption, distribution, and excretion.

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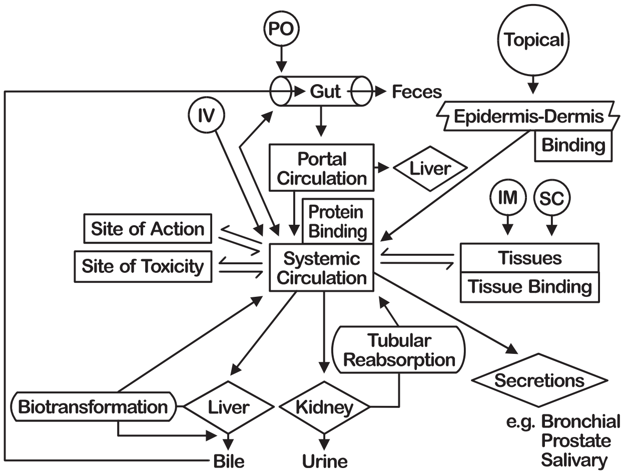


Figure 2.1 Basic schema by which drug is absorbed, distributed, metabolized, and excreted from the body. These processes are those that form the basis for developing pharmacokinetic models.

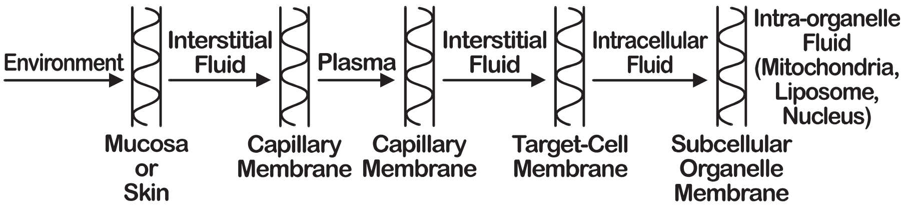
These membrane barriers often directly or indirectly define the nature of compartments or other mathematical modules in pharmacokinetic models. Biological spaces are defined by the restrictions on drug movement imposed by these barriers. The most effective barriers are those that protect the organism from the external environment. These include the skin as well as various segments of the gastrointestinal and respiratory tract, which also protect the internal physiological milieu from the damaging external environment. However, the gastrointestinal and respiratory barriers are modified deep within the body to allow for nutrient and gas exchange vital for life. The interstitial fluid is a common compartment through which any drug must transit, either after absorption on route to the blood stream or after delivery by blood to a tissue on route to a cellular target. Capillary membranes interfacing with this interstitial fluid compartment are relatively porous due to the fenestrae that allow large molecules to exchange between tissues and blood. Membranes define homogeneous tissue compartments and membranes must be traversed in all processes of drug absorption and disposition.

All cellular membranes appear to be primarily lipid bilayers into which are embedded proteins that may reside on either surface (intra- or extracellular) or traverse the entire structure. The lipid leaflets are arranged with hydrophilic (polar) head groups on the surface and hydrophobic (nonpolar) tails forming the interior. The specific lipid composition varies widely across different tissues and levels of biological organization. The location of the proteins in the lipid matrix is primarily a consequence of their hydrophobic regions residing in the lipid interior and their hydrophilic and ionic regions occupying the surface. This is thermodynamically the most stable configuration. Changes in the fluidity of the lipids alter protein conformations, which then may modulate their activity. This was one of the mechanisms of action proposed for gaseous anesthetics, although recently specific protein receptors have now been suggested. In some cases, aqueous channels form from integral proteins that traverse the membrane. In other cases, these integral proteins may actually be enzymatic transport proteins that function as active or facilitative transport systems. The primary pathway for drugs to cross these lipid membranes is by passive diffusion through the lipid environment.

Thus, in order for a drug to be absorbed or distributed throughout the body, it must be able to pass through a lipid membrane on some part of its sojourn through the body. In some absorption sites and in many capillaries, fenestrated pores exist, which allow some flow of small molecules. This is contrasted to some protected sites of the body (e.g., brain, cerebral spinal fluid) where additional membranes (e.g., glial cells) may have to be traversed before a drug arrives at its target site. These specialized membranes could be considered a general adaptation to further shelter susceptible tissues from hostile lipophilic chemicals. In this case, drug characteristics that promote transmembrane diffusion would favor drug action and effect (again unless specific transport systems intervene).

This general phenomenon of the enhanced absorption and distribution of lipophilic compounds is a unifying tenet that runs throughout the study of drug fate. The body's elimination organs can also be viewed as operating along a somewhat similar principle. The primary mechanism by which a chemical can be excreted from the body is by becoming less lipophilic and more hydrophilic, the latter property being required for excretion in the aqueous fluids of the urinary or biliary systems. When a hydrophilic or polar drug is injected into the bloodstream, it will be minimally distributed and rapidly excreted by one of these routes. However, if a compound's lipophilicity evades this easy excretion, the

Figure 2.2 Illustration of how absorption, distribution, and excretion is essentially a journey of the drug through various lipoidal membrane barriers.



liver and other organs may metabolize it to less lipophilic and more hydrophilic metabolites that have a restricted distribution (and thus reduced access to sites for activity) in the body and can be more readily excreted. This basic tenet runs throughout all aspects of pharmacology and is a useful concept to predict effects of unknown compounds.

Drug Passage Across Membranes

Considerable evidence exists that lipid-based membranes are permeable to nonpolar lipid-soluble compounds and polar water-soluble compounds with sufficient lipid solubility to diffuse through the hydrophobic regions of the membrane. The rate of diffusion of a compound across a membrane is directly proportional to its concentration gradient across the membrane, lipid\water partition coefficient, and diffusion coefficient. This can be summarized by Fick's law of diffusion in Equation 2.1:

$$\begin{array}{l}\text{Rate of}\\\text{diffusion}\\\text{(mg/ sec)}\end{array} = \frac{\text{D (cm/sec) P}}{\text{h (cm)}}\left(\text{X}_1 - \text{X}_2\right)\text{(mg)} \qquad (2.1)$$

where D is the diffusion coefficient for the specific penetrant in the membrane being studied, P is the partition coefficient for the penetrant between the membrane and the external medium, h is the thickness or actual length of the path by which the drug diffuses through the membrane, and $X_1 - X_2$ is the concentration gradient (ΔX) across the membrane. The diffusional coefficient of the drug is a function of its molecular size, molecular conformation and solubility in the membrane milieu, and degree of ionization. The partition coefficient is the relative solubility of the compound in lipid and water that reflects the ability of the penetrant to gain access to the lipid membrane. Depending on the membrane, there is a functional molecular size and/or weight cut-off that prevents very large molecules from being passively absorbed across any membrane. When the rate of a process is dependent upon a rate constant (in this case [DP/h] often referred to as the permeability coefficient P) and a concentration gradient, a linear or first-order kinetic process is evident (see Chapter 3 for full discussion). In membrane transfer studies, the total flux of drug across a membrane is dependent on the area of membrane exposed; thus the rate above is often expressed in terms of cm^2. If the lipid : water partition coefficient is too great, depending on the specific membrane, the compound may be sequestered in the membrane rather than traverse it.

Evidence also exists that membranes are more permeable to the nonionized than the ionized form of weak organic acids and bases. If the nonionized moiety has a lipid : water partition coefficient favorable for membrane penetration, it will ultimately reach equilibrium on both sides of the membrane. The ionized form of the drug is completely prevented from crossing the membrane because of its low lipid solubility. The amount of the drug in the ionized or nonionized form depends upon the pKa (negative logarithm of the acidic dissociation constant) of the drug and the pH of the medium on either side of the membrane (e.g., intracellular versus extracellular fluid; gastrointestinal versus extracellular fluid). Protonated weak acids are nonionized (e.g., COOH) while protonated weak bases are ionized (e.g., $\text{NH}_3{}^+$). If the drug has a fixed charge at all pHs encountered inside and outside of the body (e.g., quarternary amines, aminoglycoside antibiotics), they will never cross lipid membranes by diffusion. This would restrict both their absorption and distribution and generally lead to an enhanced rate of elimination. It is the nonionized form of the drug that is governed by Fick's Law of Diffusion and described by Equation 2.1 above. For this equation to predict the movement of a drug across membrane systems in vivo, the relevant pH of each compartment must be considered relative to the compound's pKa; otherwise, erroneous predictions will be made.

When the pH of the medium is equal to the pKa of the dissolved drug, 50% of the drug exists in the ionized state and 50% in the nonionized, lipid-soluble state. The ratio of nonionized to ionized drug is given by the Henderson–Hasselbalch equation (Equations 2.2 and 2.3).For acids:

$$\text{pKa} - \text{pH} = \log\left[(\text{HAcid})^0/(\text{HAcid})^-\right] \qquad (2.2)$$

For bases:

$$\text{pKa} - \text{pH} = \log\left[(\text{H Base})^+/(\text{H Base})^0\right] \qquad (2.3)$$

These equations are identical as they involve the ratio of protonated (H) to nonprotonated moieties. The only difference is that for an acid, the protonated form $(\text{H Acid})^0$ is neutral while for a base, the protonated form $(\text{H Base})^+$ is ionized. This topic is also presented in Chapter 5 (Equations 5.1 to 5.5; Figure 5.3).

As can be seen by these equations, when the pH is one unit less or one unit more than the pKa for weak bases or acids, respectively, the ratio of ionized to nonionized is 10. Thus each unit of pH away from the pKa results in a tenfold change in this ratio. This phenomenon allows for a drug to be differentially distributed across a membrane in the presence of a pH gradient, an effect that often dwarfs that obtainable by increasing dose to increase drug delivery to a specific tissue. The side of the membrane with the pH favoring ionized drug (high pH for an acidic drug; low pH for an alkaline drug) will tend to have higher total (ionized plus nonionized) drug concentrations. This pH partitioning results in so-called "ion trapping" in the area where the ionized drug predominates. Figure 2.3 illustrates this concept with an organic

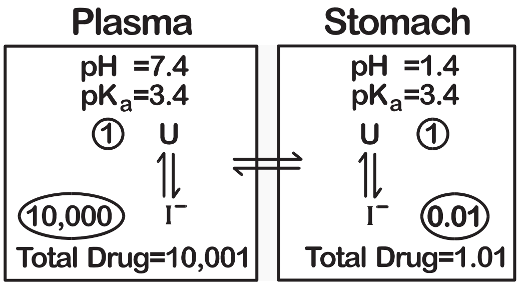


Figure 2.3 The phenomenon of pH partitioning and ion trapping of a weak acid.

acid of pKa = 3.4 partitioning between gastric contents of pH = 1.4 and plasma of pH = 7.4. Assuming that the nonionized form of the drug (U) is in equilibrium across the membrane, then, according to Equations 2.1 and 2.2, there will be a 100-fold (log 2; 3.4–1.4) difference on the gastric side and a 10,000-fold (log 4; 7.4–3.4) difference on the plasma side of the membrane, for a transmembrane concentration gradient of total drug (U plus I) equal to 10,001/1.01. Note that the unionized concentration on both sides of the membrane are in equilibrium. It is the total drug concentrations that are different. In this case, the gradient is generated by the difference in pH across an ion-impermeable barrier generated by the local milieu.

Such a gradient would greatly favor the absorption of this weak acid across the gastrointestinal tract into plasma. This is the situation that exists for weak acids such as penicillin, aspirin, and phenylbutazone. In contrast, a weak base would tend to be trapped in this environment and thus minimal absorption would occur. Examples of such weak bases are morphine, phenothiazine, and ketamine. Specific active transport systems may counter these predictions (e.g., β-lactam transporters in intestines), as well as the extreme surface area of the small intestines compared to gastric mucosa, which generally favors absorption of most drugs in the small intestines. With the weakly basic strychnine, pH-dependent absorption is toxicologically significant. If strychnine were placed into the strongly acidic stomach, no systemic toxicity would be observed. However, if the stomach were then infused with alkali, most of this base would become nonionized, readily absorbed, and lethal. In summary, weak acids are readily absorbed from an acid environment and sequestered in an alkaline medium. In contrast, weak bases are absorbed in an alkaline environment and trapped in an acidic environment.

This pH partitioning phenomenon is not only important in understanding absorption (as illustrated above), but also in any situation where the pH of fluid compartments across a biological membrane is different. It will occur for a drug distributing from plasma (pH = 7.4) to milk (pH = 6.5–6.8), to cerebrospinal fluid (pH = 7.3), or to intracellular sites (pH = 7.0). Thus weakly acidic drugs will tend not to distribute into the milk after systemic distribution (e.g., penicillin), while weakly basic drugs (e.g., erythromycin) will. If a disease process alters the pH of one compartment (e.g., mastitis), the normal equilibrium ratio will also be perturbed. In mastitis, where pH may increase almost one unit, this preferential distribution of basic antibiotics will be lost. The relatively acidic pH of cells relative to plasma is responsible for the relatively large tissue distribution seen with many weakly basic drugs (e.g., morphine, amphetamine). Similarly, in the ruminant, many basic drugs tend to distribute into the rumen, resulting in distribution volumes much larger than those in monogastrics. In fact, a drug that distributes into this organ may then undergo microbial degradation resulting in its elimination from the body.

This phenomenon is also very important for the passive tubular reabsorption of weak acids and bases being excreted by the kidney. For carnivores with acidic urine relative to plasma, weak acids tend to be reabsorbed from the tubules into the plasma while weak bases tend to be preferentially excreted. This principle has been applied to the treatment of salicylate (weak acid) intoxication in dogs where alkaline diuresis promotes ion trapping of the drug in the urine and hence its rapid excretion. Disease-induced changes in urine pH will likewise alter the disposition of drugs sensitive to this phenomenon.

Movement across the fenestrated capillaries of the body from plasma to tissue areas generally allows movement of most drugs. In these cases, relatively small molecules (molecular weights <1,000) can pass through independent of their lipid solubility, but larger molecules are excluded. In all of these scenarios, drugs move through these tissues as a solute dissolved in water and essentially are transported wherever the water goes. This process is termed *bulk flow* and is dependent on the concentration of drug dissolved in the plasma or tissue fluid. This is a linear process and thus is easily modeled by most pharmacokinetic systems. It is the subsequent uptake into cells and special tissue areas that are governed by the diffusion processes above.

There exist several specialized membranes that possess specific transport systems. In these cases, the laws of diffusion and pH partitioning do not govern transmembrane flux of drugs. These specializations in transport can best be appreciated as mechanisms by which the body can exert control and selectivity over the chemicals that are allowed to enter the protected domain of specific organs, cells, or organelles. Such transport systems can be rather nonspecific, as are those of the kidney and liver that excrete charged waste products.

In the gastrointestinal tract, relatively nonspecific transport systems allow for the absorption, and thus entrance to the body, of essential nutrients that do not have sufficient lipophilicity to cross membranes by diffusion. In specific tissues, they allow for select molecules to enter cells depending upon cellular needs, or allow compounds that circulate throughout the body to have a biological response only in a tissue possessing the correct transport receptor. The primary example is the protein carrier-mediated processes of active transport or facilitated diffusion. These systems are characterized by specificity and saturability. In the case of active transport, biological energy is utilized to move a drug against its concentration gradient. In facilitated diffusion, the carrier protein binds to the drug and carries it across the membrane down its concentration gradient. The drugs transported by these systems normally cannot cross the membrane by passive diffusion because they are not lipophilic. These systems are important for the gastrointestinal absorption of many essential nutrients and some drugs (e.g., β-lactams), for cellular uptake of many compounds (e.g., glucose), for the removal of drugs from the cerebral spinal fluid through the choroid plexus, and for biliary and renal excretion of numerous drugs.

In some tissues, cells may absorb drugs by endocytosis or pinocytosis, processes where a compound binds to the surface of the membrane that then invaginates and interiorizes the compound. This is not a primary mechanism of transmembrane passage for most therapeutic drugs. It is the primary mechanism by which nanomaterials enter cells. Most inorganic ions such as sodium and chloride are sufficiently small that they easily can cross aqueous pores and channels through membranes. The movement of these charged substances is generally governed by the transmembrane electrical potential maintained by active ion pumps.

Finally, active transport may also occur in the opposite direction to remove a drug after it has been absorbed into specific cells or tissue sites. This is called the *P-glycoprotein system,* a class of drug transporters originally associated with multiple drug resistance (MDR) encountered in cancer chemotherapy. MDR transporters have been identified in intestinal epithelial cells, the placenta, kidney tubules, brain endothelial cells, and liver bile canaliculus. These will be addressed throughout this text for specific drug classes.

In conclusion, an understanding of the processes that govern the movement of drugs across lipid-based biological membranes is important to the study of drug absorption, distribution, and excretion. Lipid-soluble drugs are easily absorbed into the body and well distributed throughout the tissues. In contrast, hydrophilic drugs are not well absorbed and have limited distribution but are easily eliminated. Metabolism converts lipophilic drugs to more easily excreted hydrophilic entities. If membranes separate areas of different pH, concentration gradients may form due to pH partitioning or ion trapping. Membranes are the building blocks of biological systems and play a central role in defining the complexity of pharmacokinetic models.

Absorption

Absorption is the movement of the drug from the site of administration into the blood. There are a number of methods available for administering drugs to animals. The primary routes of drug absorption from environmental exposure in mammals are gastrointestinal, dermal, and respiratory. The first two are also used as routes of drug administration for systemic effects, with additional routes including intravenous, intramuscular, subcutaneous, or intraperitoneal injection. Other variations on gastrointestinal absorption include intraruminal, sublingual, and rectal drug delivery. Many techniques are also used for localized therapy, which may also result in systemic drug absorption as a side effect. These include, among others: topical, intramammary, intraarticular, subconjunctival, and spinal fluid injections. Methods of utilizing these different routes of drug administration are also explored in Chapter 5.

Gastrointestinal Absorption

One of the primary routes of drug administration is oral ingestion of a pill or tablet that is designed to deliver a drug across the gastrointestinal mucosa. The common factor in all forms of oral drug administration is a method to deliver a drug such that it gets into solution in the gastrointestinal fluids from which it can then be absorbed across the mucosa and ultimately reach the submucosal capillaries and the systemic circulation. Examples of oral drug delivery systems include solutions (aqueous, elixirs) and suspensions, pills, tablets, boluses for food animals, capsules, pellets, and sustained release mechanical devices for ruminants. The major obstacle encountered in comparative and veterinary medicine is the enormous interspecies diversity in comparative gastrointestinal anatomy and physiology, which results in major species differences in strategies for and efficiency of oral drug administration. This is often appreciated but overlooked when laboratory animal data is extrapolated to humans. Rats and rabbits are widely utilized in preclinical disposition and toxicology studies, although many investigators fail to appreciate that these species' gastrointestinal tracts are very different from one another and from humans.

From a pharmacologist's perspective, the gastrointestinal tract of all species can be simply presented as diagrammed in Figure 2.4. The GI tract is best conceptualized as actually being part of the external environment, which, in contrast to the skin, is protected and whose

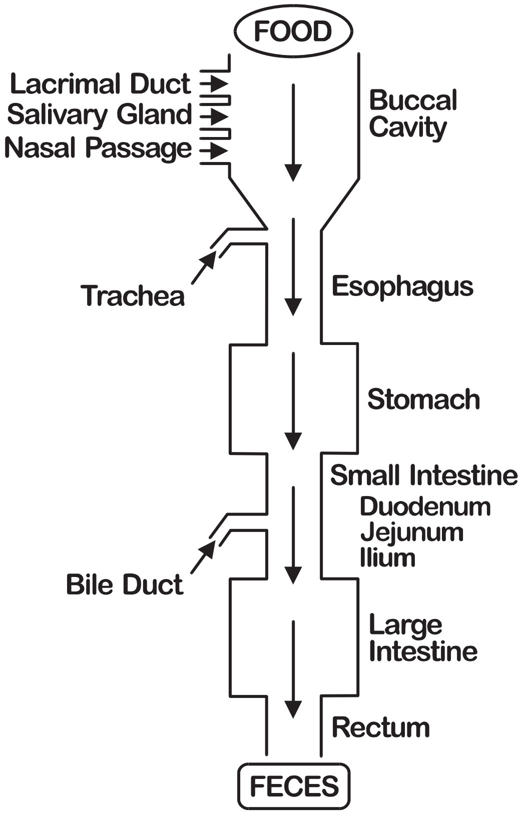


Figure 2.4 Functional structure of the gastrointestinal tract.

microenvironment is closely regulated by the organism. Because of the gastrointestinal tract's central role in digestion and nutrient absorption, there are many evolutionary adaptations to this basically simple mucosa structure that allow for physical, chemical, enzymatic, and microbial breakdown of food for liberation and ultimate absorption of nutrients. This tract is further adapted such that these digestive processes do not harm the organism's own tissues, which in carnivores may be identical to the food being eaten.

The gastrointestinal tract presents a significant degree of heterogeneity relative to morphology and physiology that translates to great regional variations in drug absorption. In the oral cavity, where food is masticated, some absorption may occur in sublingual areas. In fact, this site is actually utilized as a route for systemic drug (e.g., nitroglycerin) and nicotine (e.g., oral tobacco) delivery in humans. The esophagus and cranial portion of the stomach is lined by cornified epithelium, which provides an effective barrier that often decreases the chance of absorption for drugs formulated for intestinal drug delivery. A great deal of recent research activity has been focused on developing new transbuccal drug delivery systems. As mentioned, the prototype example was sublingual nitroglycerin tablets. Newer systems use novel adhesive technology, which allow actual polymer patches to adhere to the buccal mucosa. Such products are also being considered for some therapeutic applications in veterinary medicine (e.g., feline oral sprays). Compared to oral gastrointestinal absorption, buccal delivery bypasses the portal vein and thus eliminates the potential for first-pass hepatic biotransformation, discussed later.

The simple mucosal lining of the stomach allows absorption; however, the presence of surface mucus, which protects the epithelium from self-digestion secondary to acid and enzyme secretion, may be a barrier for some drugs. The acidity and motility of the stomach also creates a hostile environment for drugs and even influences the absorption of drugs farther down the tract. For oral drug absorption to be successful, the drug must be capable of surviving this relatively harsh environment. For some drugs (e.g., penicillin G) susceptible to acid hydrolysis, minimal absorption by the oral route will occur unless they are administered in a formulation that protects them in an acid environment but liberates them in the more alkaline environment of the intestines. Release of the drug from the stomach, a process controlled by gastric emptying, is a major rate-determining step in the onset and duration of oral drug activity. Species differences in the size of the pyloric orifice also limit use of some dosage forms in small animals versus humans.

The primary site for most drug absorption is the small intestine. In this region of the gastrointestinal tract, the pH of the contents are more alkaline and the epithelial lining is conducive to drug absorption. The blood flow to this region is also much greater than to the stomach. The small intestine is lined by simple columnar epithelium resting on a basement membrane and a submucosal tissue bed that is very well perfused by an extensive capillary and lymphatic network. This capillary bed drains into the hepatic portal vein. One of the major anatomical adaptations for absorption in this region is the presence of microvilli, which increase the surface area of the small intestine some 600-fold over that of a simple tube. The second anatomical adaptation are the villi of the intestine, which can be easily appreciated by examining a cross section (Figure 2.5). Since diffusion is the primary mechanism for drug absorption, the increase in area due to these two anatomical configurations significantly increases absorption, as can be seen from reviewing the area contribution to Equation 2.1. There are species differences in inherent permeability of the intestinal mucosa to chemicals, with the dog recently being recognized as having a higher permeability to many drugs than humans.

The viable epithelial cells of the intestines are also endowed with the necessary enzymes for drug metabolism that contributes to a second "first-pass" effect. Recent research has also indicated that the mechanism and extent of absorption, and magnitude of local

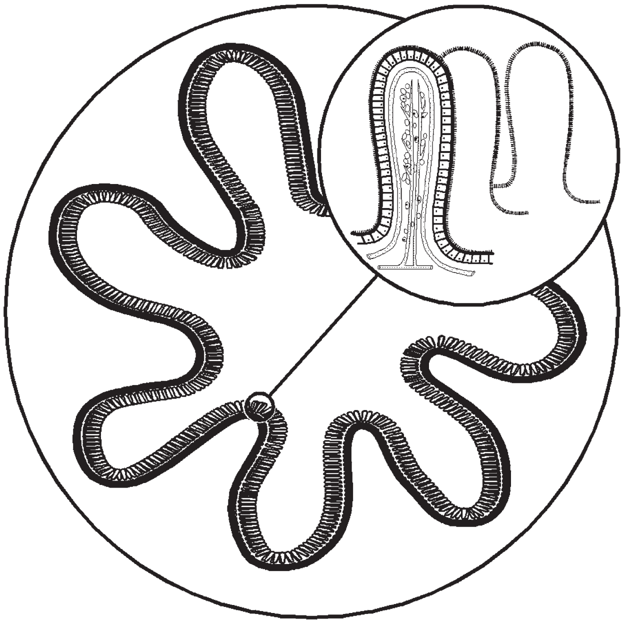


Figure 2.5 Cross-section of the small intestine showing villi adaptations, which serve to increase surface area available for absorption.

intestinal metabolism, varies between the tips and crypts of the villi. The final determinant of a drug's tortuous journey through the gastrointestinal tract is the resident microbial population that inhabits the intestinal contents. Many bacteria are capable of metabolizing specific drugs, resulting in a third component of the first-pass effect. This epithelial and bacterial biotransformation is generally categorized as *presystemic* metabolism to differentiate it from that which occurs following portal vein delivery of drug to the liver. However, from the perspective of pharmacokinetic analysis of plasma drug concentrations following oral drug administration, all three components are indistinguishable and become lumped into the aggregate process of oral absorption assessed as K_a.

Disintegration, Dissolution, Diffusion, and Other Transport Phenomenon

In order for a drug to be absorbed across the intestinal mucosa, the drug must first be dissolved in the aqueous intestinal fluid. Two steps, disintegration and dissolution, may be required for this to occur. *Disintegration* is the process whereby a solid dosage form (e.g., tablet) physically disperses so that its constituent particles can be exposed to the gastrointestinal fluid. Dissolution occurs when the drug molecules then enter into solution. This component of the process is technically termed the *pharmaceutical phase* and is controlled by the interaction of the formulation with the intestinal contents. These concepts are further elaborated on in Chapter 5.

Some dosage forms, such as capsules and lozenges, may not be designed to disintegrate, but rather to allow a drug to slowly elute from their surface. Dissolution is often the rate-limiting step controlling the absorption process and can be enhanced by formulating the drug in salt form (e.g., sodium or hydrochloride salts), buffering the preparation (e.g., buffered aspirin), or decreasing dispersed particle size (micronization) so as to maximize exposed surface area. This is extensively discussed in Chapter 5 (see equation 5.7). Alternatively, disintegration and dissolution can be decreased so as to deliberately provide slow release of the drug. This strategy is used in *prolonged-release* or *controlled-release* dosage forms and involves complex pharmaceutical formulations that produce different rates of dissolution. This may be accomplished by dispersing the dosage form into particles with different rates of dissolution or by using multilaminated dosage forms, which delays release of the drug until its layer is exposed. All of these strategies decrease the overall rate of absorption. Similar strategies can also be used to target drugs to the distal segments of the gastrointestinal tract by using enteric coatings that dissolve only at specific pH ranges, thereby preventing dissolution until the drug is in the region targeted. This strategy has been applied for colonic delivery of drugs in humans for treatment of Crohn's disease.

In slow-release or long-acting formulations, the end result is that absorption becomes slower than all other distribution and elimination processes, making the pharmaceutical phase the rate-limiting or rate-controlling step in the subsequent absorption and disposition of the drug. When this occurs, as will be seen in the pharmacokinetic modeling chapters to follow, the rate of absorption controls the rate of apparent drug elimination from the body and a so-called *flip-flop* scenario becomes operative.

There are significant species differences in the ability to use controlled-release oral medications designed in humans, by far the largest market, in other species. The first limitation involves the inability to use cellulose-based systems in ruminants due to the ability of rumen microbes to digest the normally inert cellulose matrix that controls rates of drug delivery. The second arises because of shorter gastrointestinal transit times in small carnivores, such as domestic cats and dogs, compared to humans. In this instance, drug release is designed to occur in the longer transit times seen in humans (approx. 24 hours). In dogs and cats, which have transit times half that of humans, drug release may still be occurring even after the tablet has been eliminated in the feces due to the shorter transit times. Other examples include the narrower pyloric opening in dogs, compared to humans, that may increase gastric retention of some larger dosage forms. These are but a few examples of significant species differences that, based on anatomical and physiological factors, prevent the ready transferability of complex dosage forms across species.

After the drug is in solution, it must still be in a nonionized relatively lipid-soluble form to be absorbed across the lipid membranes comprising the intestinal mucosa. *It must be stressed that absorption across any membrane is a fine balance between adequate solubility on the donor side of the membrane with sufficient permeability (or active transport capacity) to actually transit the membrane.* For orally administered products, the pH of the gastrointestinal contents becomes very important, as is evident from the earlier discussion on pH partitioning. Specifically, a weak acid would tend to be preferentially absorbed in the more acidic environment of the stomach since a larger fraction would be in the nonionized form. However, the much larger surface area and blood flow available for absorption in the more alkaline intestine may override this effect. It is important to mention at this point why a weak acid such as aspirin is better absorbed in a bicarbonate buffered form, which would tend to increase the ionized fraction and thus decrease membrane passage. The paradox is that dissolution must first occur, a process favored by the ionized form of the drug. It is only the dissolved ionized aspirin that is available to the partitioning phenomenon described earlier. Thus, when more aspirin is dissolved in the buffered microenvironment, more is available for partitioning and diffusion across the mucosa. In contrast to the situation of a weak acid, a weak base would tend to be better absorbed in the more alkaline environment. However, it must be repeated that the very large surface area available in the intestines, coupled with high blood flow and a pH of approximately 5.3 in the immediate area of the mucosal surface makes it the primary site of absorption for most drugs (weak acids with pKa >3 and weak bases with pKa <7.8). Species differences in both gastric and intestinal pH further modulate this differential (e.g., canine gastric pH is much higher than humans). A further obstacle to absorption is that the compound must also be structurally stable against chemical or enzymatic attack. Finally, compounds with a fixed charge and/or very low (or very high) lipid solubility for the uncharged moiety, may not be significantly absorbed after oral administration. Examples include the polar aminoglycoside antibiotics, the so-called "enteric" sulfonamides, and quaternary ammonium drugs.

There are also specific active transport systems present within the intestinal mucosa of the microvilli that are responsible for nutrient absorption. However, these systems have a very high capacity and if a specific drug or toxicant has the proper molecular configuration to be transported, saturation is unlikely. There is some evidence that select therapeutic drugs (e.g., β-lactams such as ampicillin) may be absorbed by active transport systems in the small intestine. There are also transport systems (P-glycoprotein) that expel absorbed drug back into the intestinal lumen. This system is beginning to be studied more closely in veterinary species and will be discussed later in this chapter under distribution and elimination.

The complexity of these processes is clearly seen when one tries to extrapolate oral bioavailability of drugs between dogs and humans. In order to try and classify drug absorption in humans based on criteria of solubility and permeability, the Biopharmaceutical Classification System (BCS) was developed, which ranked drugs according to their extent and rate of absorption, with Class I drugs being both highly soluble and permeable resulting in generally very well absorbed compounds, in contrast to Class IV which had low solubility and low permeability and showed very poor oral absorption. Class II and III have mixed solubility and permeability characteristics. The advantage of such classifications is that manufactures changing formulations for Class I drugs only need to conduct in vitro dissolution studies since dissolution would be the rate-limiting process in absorption. Class IV drugs would require in vivo comparisons. Drugs classified using this system for humans did not correlate to what was observed in dogs, suggesting that the differences in GI physiology discussed above prevents easy interspecies extrapolations (Papich and Martinez, 2015).

Enterohepatic Recycling and Coprophagy

The gastrointestinal tract has also evolved into an excretory organ for elimination of nonabsorbed solid wastes and other metabolic byproducts excreted in the bile. The bile duct drains into the upper small intestine. For some drugs, this results in a phenomenon called *enterohepatic recycling* whereby a drug from the systemic circulation is excreted into the bile and is reabsorbed from the small intestine back into the bloodstream. In many cases, drugs that are metabolized by Phase II conjugation reactions are "unconjugated" by resident bacterial flora, which generates free drug for reabsorption. Thus compounds that are excreted into the bile may have a prolonged sojourn in the body because of the continuous opportunity for intestinal reabsorption. The cardinal sign of this process is a "hump" in the plasma drug concentration-time profile after administration (Figure 2.6). Bile also serves to emulsify fatty substances that are not capable of solubilizing in the primarily aqueous environment of the intestines. The result of this detergent-like action of bile is to form large surface area micelles having a hydrophilic surface and hydrophobic interior. These act as transport vehicles to deliver fat-soluble drugs to the intestinal brush border surface for diffusion across the lipid membrane into the cell. Without the interaction of bile acids, fatty substances would not be available for absorption since they could not traverse this "dissolution" barrier. Thus unlike most drugs, compounds that are absorbed

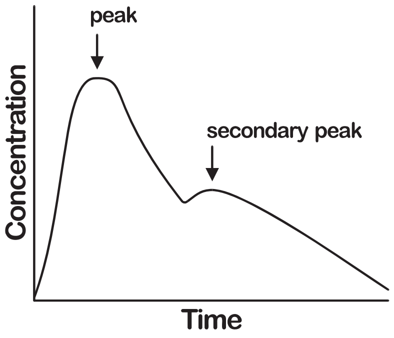


Figure 2.6 Concentration versus time profile demonstrating a secondary peak that could result from enterohepatic recycling.

by this route often must be administered with a meal to promote bile acid secretion and associated micelle formation.

Enterohepatic recycling has also been suggested as an important mechanism for enhanced activity of certain antiparasitic drugs such as the avermectins. As discussed in Chapter 41, the recycling of active drug is a major contributor to enhanced parasite exposure. Finally, some species such as rabbits, routinely ingest fresh feces for nutritional purposes, which provides another opportunity for drug to be reabsorbed into the body.

Species Effects on Gastrointestinal Transit Time and Food Interactions

Food may also interact with other aspects of oral drug absorption and have opposite effects for more hydrophilic drugs. Depending on the physicochemical properties of the specific drug, administration with food may significantly increase or decrease absorption. Such effects are not only drug dependent, but also are species dependent due to the continuous foraging behavior of ruminants and some other omnivores compared to the periodic feeding habits of predatory carnivores. These variables are difficult to incorporate into formal pharmacokinetic models yet they add to the variability in parameters derived from these studies or in drug response between species.

The first potential interaction relates to the rate of drug delivery to the small intestine that is governed by the rate of drug release from the stomach, the *gastric emptying time*. This process is dependent upon the eating habits of the species, with continuous foraging animals (e.g., herbivores such as horses and ruminants) having a steady input of drug and a relatively stable gastric pH compared to periodic eaters (e.g., carnivores like dogs and cats and omnivores such as pigs) who have more variable eating patterns with large swings in gastric pH depending on the presence or absence of food. In addition, the drug may directly interact with the ingested food, as is the case of chelation of tetracyclines with divalent cations such as Mg^{++} in antacids or Ca^{++} in milk products. Thus, the decision to administer a compound with or without food is species and drug dependent and may significantly alter the bioavailability (rate and extent of absorption) of the drug. In contrast, for very-lipid-soluble drugs, food is necessary in order to have bile release, which allows solubilization and absorption to occur.

The forestomachs of a ruminant provide a major obstacle to the delivery of an oral dosage form to the true stomach (abomasum) for ultimate release to the intestines, although a significant amount of drug absorption may occur from this site. The rumen is essentially a large fermentation vat (>50 liters in cattle, 5 liters in sheep) lined by stratified squamous epithelium, buffered at approximately a pH of 6 by extensive input of saliva, which maintains it in a fluid to soft consistency, designed primarily for the absorption of volatile fatty acids. If drugs dissolve in this medium and remain intact, they undergo tremendous dilution that decreases their rate of absorption. They then are pumped from the rumen and reticulum through the omasum for a rather steady input of drug into the true stomach. An understanding of the physiology of the ruminant has allowed for the development of some unique and innovative mechanical drug delivery technologies, which essentially are encapsulated pumps that “sink” to the bottom of the rumen and become trapped, much as many unwanted objects tend to when ingested by a ruminant (e.g., nails and wire in hardware disease). These “submarine-like” devices then slowly release drug into the ruminal fluid for a true sustained-release preparation. In preruminant calves, a drug may bypass the rumen entirely through the rumen-reticulo groove and essentially behave as if administered to a monogastric. In contrast, fermentation in the horse occurs after drug absorption by the small intestine and thus has less impact than in ruminants. However, a nonabsorbed drug that reaches the equine large intestines and cecum, the site of fermentation, may have disastrous effects (e.g., colic) if digestive flora or function is perturbed.

First-Pass Metabolism

Another unique aspect of oral drug absorption is the fate of the absorbed drug once it enters the submucosal capillaries. Drug absorbed distal to the oral cavity and proximal to the rectum in most species enters the portal circulation and is transported directly to the liver where biotransformation may occur. This is a major cause for differences in a drug’s ultimate disposition compared to all other routes of administration. This may result in a significant first-pass biotransformation of the absorbed compound. For a drug that is extensively metabolized by the liver, this first-pass effect significantly reduces absorption of the active drug even when it is absorbed across the mucosa. This occurs for many opiate medications in dogs, reducing their efficacy after oral administration. Finally, some drugs that are too polar to be absorbed across the gastrointestinal wall are formulated as ester conjugates to increase lipid solubility and enhance absorption. Once the drug crosses the gastrointestinal epithelium in this form, subsequent first-pass hepatic biotransformation enzymes and circulating blood and mucosal esterases cleave off the ester moiety releasing free drug into the systemic circulation.

There are selected drug administration sites that avoid first-pass hepatic metabolism by allowing absorption through gastrointestinal tract segments *not* drained by the portal vein. These include the oral cavity buccal and rectal routes of drug administration in some species, although this assumption hasn't been tested in many veterinary species.

Formulation Factors

The pharmaceutical literature is replete with formulation factors that may influence the dissolution and absorption of a drug preparation, assuming in the first place that one has an active component of known purity and potency. These are fully discussed in Chapter 5. The issue then becomes what are the potential interactions that can occur between the active ingredients and the excipients that make up the formulation. Additionally, what are the effects of the practitioner's compounding techniques (materials used, mixing efficacy, etc.) on the amount of active ingredients ultimately appearing in the formulation. Although this discussion is the focus of a biopharmaceutics text, the strategies are often encountered in pharmacokinetics as they may affect the parameters estimated after oral administration.

Table 2.1 depicts the pharmaceutical processes involved in absorption that may be affected by formulation. Following oral administration of tablets, disintegration must first occur. The speed and efficacy of this process will determine how much drug is actually available for subsequent steps. The resulting particle size (and hence surface area) is an important determinant for the next dissolution phase where the drug enters solution, an absolute prerequisite for diffusion across the mucosal barrier. Dissolution also involves diffusion across the liquid boundary layers that are an interface between the particles and the absorption milieu. Many pharmaceutical factors may affect the efficiency of the disintegration and dissolution processes. For tablets, the nature and homogeneity of the excipients become important considerations. These factors are the primary determinants of differences in efficacy between so-called "pioneer" and generic drug products. Once the drug is in solution, binding or complexation to inert filler ingredients may occur. It is important to remember that all of this is happening while the particles are in transit through the gastrointestinal tract. Thus, if the formulation results in a decreased rate of disintegration or dissolution, the rate and extent of absorption may be decreased, especially in species with very short gastrointestinal transit times. Similar factors are involved with oral capsules and even liquid dosage forms where the drug may interact with the vehicle. In fact, these scenarios are probably most pertinent to practitioner compounding. For capsules, the breakdown of the capsule replaces tablet disintegration as the initial rate-determining step. After release of the capsule contents, all of the above factors come into play. It cannot be overstated that such pharmaceutical factors are critical determinants of the extent and rate of subsequent drug absorption.

Table 2.1 Pharmaceutical factors affecting absorption

- Disintegration
  - Excipients
  - Compaction pressure
  - Enteric coatings, capsules
  - Homogeneity
- Dissolution
  - Particle size/surface area
  - Binding
  - Local pH, buffers
  - Boundary layers
- Barrier diffusion
  - Solubility
  - Transit time

Topical and Percutaneous Absorption

The skin is a complex, multilayered tissue comprising 18,000 cm^2 of surface in an average human male. The quantitative prediction of the rate and extent of percutaneous penetration (into skin) and absorption (through skin) of topically applied chemicals is complicated by the biological variability inherent in skin. Mammalian skin is a dynamic organ with a myriad of biological functions. The most obvious is its barrier property that is of primary concern in the absorption problem. Another major function is thermoregulation that is achieved and regulated by three mechanisms in skin: thermal insulation provided by pelage and hair, sweating, and alteration of cutaneous blood flow. Other functions of skin include mechanical support, neurosensory reception, endocrinology, immunology, and glandular secretion. These additional biological roles lead to functional and structural adaptations that affect the skin's barrier properties and thus the rate and extent of percutaneous absorption. Many of the topics discussed below are fully developed in Chapter 47 of this text.

The skin is generally considered to be an efficient barrier preventing absorption (and thus systemic exposure) of most topically administered compounds. It is a membrane that is relatively impermeable to aqueous solutions and most ions. It is, however, permeable in varying

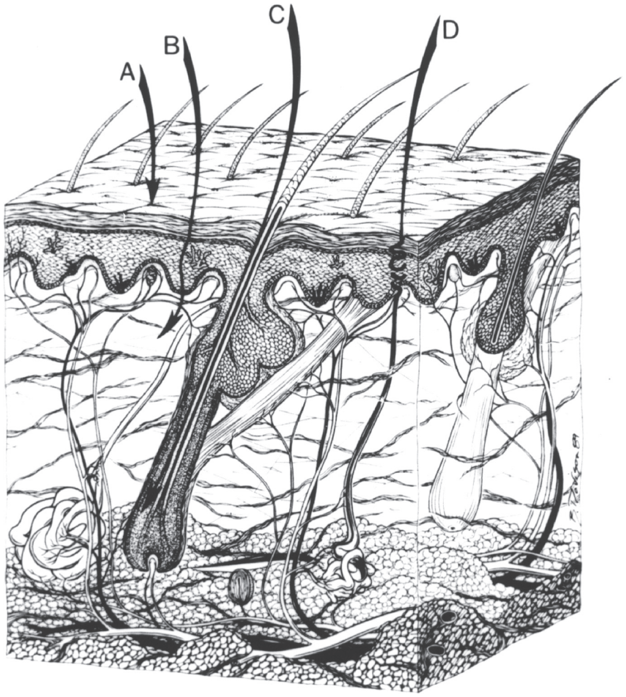


Figure 2.7 Microstructure of mammalian skin showing potential routes of penetration (A) intercellular, (B) transcellular, (C) intrafollicular, (D) via sweat ducts.

degrees to a large number of solid, liquid, and gaseous xenobiotics. Although one tends to think of most cases of poisoning as occurring through the oral or, less frequently, the respiratory route, the widespread use of organic chemicals has enhanced risk exposure to many toxicants that can penetrate the dermal barrier.

The gross features of mammalian skin are illustrated in Figure 2.7. Compared to most routes of drug absorption, the skin is by far the most diverse across species (e.g., sheep versus pig) and body sites (e.g., human forearm versus scalp). Three distinct layers and a number of associated appendages make up this nonhomogenous organ. The epidermis is a multilayered tissue varying in thickness in humans from 0.15 mm (eyelids) to 0.8 mm (palms). The primary cell type found in the epidermis is the keratinocyte. Proliferative layers of the basal keratinocyte (stratum germinativum) differentiate and gradually replace the surface cells (stratum corneum) as they deteriorate and are sloughed from the epidermis. A number of other cell types are also found interspersed in the epidermis including the pigmented melanocytes, Merkel cells which may play a sensory role, and Langerhans cells which probably play a role in cutaneous immunology.

In respect to drug penetration, the primary biochemical change is the production of fibrous, insoluble keratin that fills the cells, and a sulfur-rich amorphous protein that comprises the cell matrix and thickened cell membrane. In addition, the keratinocytes synthesize a variety of lipids that form the distinguishing granules in the stratum granulosum that release their contents into the intercellular spaces. The end result in the stratum corneum is dead proteinaceous keratinocytes embedded in an extracellular lipid matrix, a structure referred to by Elias as the “brick and mortar” model (see Figures 47.3 and 47.4).

The Stratum Corneum Barrier

It is this outermost layer, the stratum corneum, which provides the primary barrier to the penetration of foreign compounds. This barrier consists of flattened, stratified, highly keratinized cells embedded in a lipid matrix composed primarily of sterols, other neutral lipids, and ceramides. Although highly water retarding, the dead, keratinized cells are highly water absorbent (hydrophilic), a property that keeps the skin supple and soft. A natural oil covering the skin, the sebum, especially present in some species such as sheep, appears to maintain the water-holding capacity of the epidermis but has no appreciable role in retarding the penetration of xenobiotics. Disruption of the stratum corneum removes all but a superficial deterrent to penetration.

Dermis and Appendages

The dermis is a highly vascular area, providing ready access for drug distribution once the epithelial barrier has been passed. The blood supply in the dermis is under complex, interacting neural and local humoral influences whose temperature-regulating function can have an effect on distribution by altering blood supply to this area. The absorption of a chemical possessing vasoactive properties would be affected through its action on the dermal vasculature; vasoconstriction would retard absorption and increase the size of a dermal depot, while vasodilation may enhance absorption and minimize any local dermal depot formation.

The appendages of the skin are found in the dermis and extend through the epidermis. The primary appendages are the sweat glands (eccrine and apocrine), hair, and sebaceous glands, all of which show great interspecies and interregional variability. Since these structures extend to the outer surface, they potentially play a role in the penetration of certain compounds.

Topical Drug Delivery and the Definition of Dose

From the perspective of pharmacokinetic models of transdermal and topical drug delivery systems, there are significant differences from other routes of administration (e.g., oral, injection) as to what constitutes a dose. For most exposures, the concentration applied to the surface of the skin exceeds the absorption capacity. However, for therapeutic transdermal patches with a fixed concentration of the drug and rate-controlled release properties, it is the contact surface area that more accurately reflects dose and thus dose is expressed not in mg/kg, but mg/cm^2 of dosing area. This surface area dependence also holds for any topical application even if absorption capacity is superseded. Yet, another source of nonlinearity results secondary to the effects of occlusive (water-impermeable) drug vehicles or patches. As the skin hydrates, a threshold is reached where transdermal flux dramatically increases (approximately 80% relative humidity). When the skin becomes completely hydrated under occlusive conditions, flux can be dramatically increased. Therefore, dose alone is often not a sufficient metric to describe topical doses, and application method and surface area become controlling factors.

Pathways for Dermal Absorption

Anatomically, percutaneous absorption might occur through several routes. The current consensus is that the majority of nonionized, lipid-soluble toxicants appear to move through the intercellular lipid pathway between the cells of the stratum corneum, the rate-limiting barrier of the skin. Very small and/or polar molecules appear to have more favorable penetration through appendages or other diffusion shunts, but only a small fraction of drugs are represented by these molecules. Simple diffusion seems to account for penetration through the skin whether by gases, ions, or nonelectrolytes.

The rate of percutaneous absorption through this intercellular lipid pathway is correlated to the partition coefficient of the penetrant. This has resulted in numerous studies correlating the extent of percutaneous absorption with a drug's lipid : water partition coefficient. Some workers further correlated skin penetration to molecular size and other indices of potential interaction between the penetrating molecule and the skin that are not reflected in the partition coefficient. For most purposes, however, dermal penetration is often correlated to partition coefficient. If lipid solubility is too great, compounds that penetrate the stratum corneum may remain there and form a reservoir. Alternatively, penetrated compounds may also form a reservoir in the dermis. For such compounds, slow release from these depots may result in a prolonged absorption half-life. Conditions that alter the composition of the lipid (harsh delipidizing solvents, dietary lipid restrictions, disease) may alter the rate of compound penetration by changing its partitioning behavior. Very similar to the situation with oral absorption previously discussed, the actual utility of a topical drug is a delicate balance between solubility and permeability which topical drug formulators attempt to exploit. Some of these concepts are further discussed in Chapters 5 and 47.

Recent studies have demonstrated that the skin may also be responsible for metabolizing topically applied compounds. Both Phase I and II metabolic pathways have been identified. For some compounds, the extent of cutaneous metabolism influences the overall fraction of a topically applied compound that is absorbed, making this process function as an alternate absorption pathway. Cutaneous biotransformation is used to promote the absorption of some topical drugs that normally would not penetrate the skin. Cutaneous metabolism may be important for certain aspects of skin toxicology when nontoxic parent compounds are bioactivated within the epidermis, for example benzo(a)pyrene to an epoxide. Finally, resident bacteria on the surface of the skin may also metabolize topical drugs, as was demonstrated with pentochlorophenol absorption in pig skin dosed in soil with and without antibiotics. This effect is potentiated under warm and wet occlusive dosing conditions that both promote bacterial growth and reduce skin barrier properties.

Variations in Species and Body Region

Penetration of drugs through different body regions varies. In humans, generally the rate of penetration of most nonionized toxicants is in the following order: scrotal > forehead > axilla = scalp > back = abdomen > palm and plantar. The palmar and plantar regions are highly cornified producing a much greater thickness that introduces an overall lag time in diffusion. In addition to thickness, the actual size of corneacytes and differences in hair follicle density may affect absorption of more polar molecules. Finally, differences in cutaneous blood flow that have been documented in different body regions may be an additional variable to consider in predicting the rate of percutaneous absorption. These factors are also important in animals, with the area of the inner ear known to be highly permeable and well perfused, thus an excellent site for drug absorption to occur.

Although generalizations are tenuous at best, human skin appears to be more impermeable, or at least as impermeable, as the skin of the cat, dog, rat, mouse, or guinea pig. The skin of pigs and some primates serve as useful approximation to human skin, but only after a comparison has been made for each specific substance. The major determinants of species differences are thickness, hair density, lipid composition, and cutaneous blood flow.

Factors that Modulate Absorption

Soaps and detergents are perhaps the most damaging substances routinely applied to skin. Whereas organic solvents must be applied in high concentrations to damage the skin and increase the penetration of solute through human epidermis, only 1% aqueous solutions of detergents are required to achieve the same effect. For a specific chemical, rate of penetration can be drastically modified by the solvent system used. In transdermal patches, specific chemical enhancers (e.g., solvents such as ethanol; other lipid-interacting moieties) are included in the formulation to reversibly increase skin permeability and enhance drug delivery. Alternatively, drug release is formulated to be rate limiting from the patch system (membranes, microencapsulation, etc.) so that a constant (zero-order) release from the patch occurs, thereby providing controlled drug delivery. Since patches are designed with the permeability properties of specific species in mind, care must be taken when using a patch designed for one species in another. There are a number of topical veterinary drugs routinely used to achieve long-acting systemic therapeutic endpoints in animals. These include the topical pesticides formulated as "spot-ons" and "pour-ons." These products are further discussed in Chapters 43 and 47 of this text. All of these issues have also been discussed in a comprehensive review of this topic (Riviere and Papich, 2001).

Another strategy for transdermal delivery, which has not widely been employed in veterinary medicine, is to overcome the cutaneous barrier by using electrical (iontophoresis) or ultrasonic (phonophoreses) energy, rather than the concentration gradient in diffusion, to drive drugs through the skin. These techniques hold the most promise for delivering peptides and oligonucleotide drugs that now only can be administered by injection. In these cases, dose is based on the surface area of application and the amount of energy required to actively deliver the drug across skin. In iontophoresis, this amounts to a dose being expressed in μAmps/cm^2. Formulation factors are also very different since many of the excipients used are also delivered by the applied electrical current in molar proportion to the active drug. Finally, a recent but related strategy is to use very short-duration, high-voltage electrical pulses (electroporation) to reversibly break down the stratum corneum barrier, allowing larger peptides and possibly even small proteins to be systemically delivered.

Respiratory Absorption

The third major route for systemic exposure to drugs and toxicants is the respiratory system. Since this system's primary function is gas exchange (O_2, CO_2), it is always in direct contact with environmental air as an unavoidable part of breathing. A number of toxicants are in gaseous (CO, NO_2, formaldehyde), vapor (benzene, CCl_4), or aerosol (lead from automobile exhaust, silica, asbestos) forms and are potential candidates for entry via the respiratory system. There are no approved inhalational drugs for use in veterinary medicine. Each mode of inhalational exposure results in a different mechanism of compound absorption and for the purposes of this text, a different definition of dose. These concepts are also discussed In Chapters 11 and 48 of this text.

Opportunities for systemic absorption are excellent through the respiratory route since the cells lining the alveoli are very thin and profusely bathed by capillaries. The surface area of the lung is large (50–100 m^2), some 50 times the area of the skin. Based on these properties and the diffusion equation presented earlier (Equation 2.1), the large surface area, the small diffusion distance, and high level of blood perfusion maximize the rate and extent of passive absorption driven by gaseous diffusion.

The process of respiration involves the movement and exchange of air through several interrelated passages including the nose, mouth, pharynx, trachea, bronchi, and successive smaller airways terminating in the alveoli where gaseous exchange occurs. All of these anatomical modifications protect the internal environment of the air passages from the harsh outside environment by warming and humidifying the inspired air. The passages also provide numerous obstacles and baffles to prevent the inhalation of particulate and aerosol droplets. Thus the absorption of particulate and aerosolized liquids, such as those employed in nebulized drug therapy, is fundamentally different from that of gases. The absorption of such impacted solids and liquids along the respiratory tract has much more in common with oral and topical absorption, with the critical caveat that the precise dose of compound finally available for absorption is very difficult to determine. Great strides have been made in developing aerosol drug delivery devices for human use that take advantage of this mechanism of impaction; however, these may not be transferable to veterinary species since their efficacy is closely related to the geometry and physiology of the human respiratory tract.

Another unique aspect of respiratory exposure is the fact that the pulmonary blood circulation is in series with the systemic circulation. Thus, in contrast to cutaneous or oral exposure, compounds absorbed in the lung will enter the oxygenated pulmonary veins that drain to the systemic arterial circulation. Compared to oral administration, this reduces first-pass hepatic metabolism. However, the pulmonary circulation is adept at metabolizing peptides secondary to its role in inactivating peptide hormones.

Vapors and Gases

Since the rate of entry of vapor-phase toxicants is controlled by the alveolar ventilation rate, the toxicant is presented to the alveoli in an interrupted fashion whose frequency in humans is equal to the rate of breathing: about 20 times/min. Doses are generally discussed in terms of the partial pressure of the gas in the inspired air. Upon inhalation of a constant tension of a toxic gas, arterial plasma tension of the gas approaches its tension in the expired air. The rate of entry is then determined by the blood solubility of the toxicant. If there is a high blood : gas partition coefficient, a larger amount must be dissolved in the blood to raise the partial pressure. Gases with a high blood : gas partition coefficient require a longer period to approach the same tension in the blood as in inspired air than it takes for less soluble gases. Similarly, a longer period of time is required for blood concentrations of such a gas to be eliminated, thus prolonging detoxification.

Another important point to consider in determining how much of an inhaled gas is absorbed into the systemic circulation is the relationship of the fraction of lung ventilated compared to the fraction perfused. Increased perfusion of the lung will favor a more rapid achievement of blood–gas equilibrium. Decreased perfusion will decrease the absorption of toxicants even those that reach the alveoli. Various "ventilation/perfusion mismatches" may alter the amount of an inhaled gas that is systemically absorbed. Similarly, pulmonary diseases that thicken the alveoli or obstruct the airways may also affect overall absorption.

Aerosols and Particulates

The absorption of aerosols and particulates is affected by a number of physiological factors specifically designed to preclude access to the alveoli. The upper respiratory tract, beginning with the nose and continuing down its tubular elements, is a very efficient filtering system for excluding particulate matter (solids, liquid droplets). The parameters of air velocity and directional air changes favor impaction of particles in the upper respiratory system. Particle characteristics such as size, coagulation, sedimentation, electrical charge, and diffusion are important to retention, absorption, or expulsion of airborne particles. In addition to these characteristics, a mucous blanket propelled by ciliary action clears the tract of particles by directing them to the gastrointestinal system (via the glottis) or to the mouth for expectoration. This system is responsible for 80% of toxicant lung clearance. In addition to this mechanism, phagocytosis is very active in the respiratory tract, both coupled to the directed mucosal route and via penetration through interstitial tissues of the lung and migration to the lymph, where phagocytes may remain stored for long periods in lymph nodes. Compared to absorption in the alveoli, absorption through the upper respiratory tract is quantitatively of less importance. However, inhaled toxicants that become deposited on the mucous layer can be absorbed into the myriad of cells lining the respiratory tract and exert a direct toxicological response. This route of exposure is often used to deliver pharmaceutics by aerosol. If a compound is extremely potent, systemic effects may occur.

The end result of this extremely efficient filtering mechanism is that most inhaled drugs deposited in nasal or buccal mucous ultimately enter the gastrointestinal tract. This can best be appreciated by examining the respiratory drainages depicted in Figure 2.1. Therefore, the disposition of aerosols and particulates largely mirrors that of orally administered drugs.

Nasal administration is a preferred route for many inhalant medications in humans. In these cases, great care is made to deliver aerosols of the specific size for deposition on the nasal mucosa and upper respiratory tract. The bioavailability of these compounds is assessed using the techniques developed for other routes, although a local effect is often desired. The problems with this strategy are the attainment of an accurately delivered dose and the inactivation and binding of administered drug by the thick mucous blanket. Drugs delivered by this route usually have a wide therapeutic window and large safety index. The final point to consider relates to some specific peculiarities of nasal absorption. In the region of the olfactory epithelium, there exists a direct path for inhaled compounds to be absorbed directly into the olfactory neural tissue and central nervous system, thereby bypassing both the systemic circulation and the blood–brain barrier. The mass of drugs involved in this uptake process is very small and thus would not affect a pharmacokinetic analysis. However, this route has obvious toxicological significance and unfortunately has not been carefully studied in veterinary species.

Other Routes of Administration

In order to complete this discussion of absorption, it is important to realize that there are other extravascular drug administration routes that are often encountered. Relative to pharmacokinetic analysis, these are dealt with in the same fashion as the primary routes discussed above. The important difference is that in all cases, the barrier to absorption is less than that encountered in oral or topical delivery. Second, all of these routes involve an invasive procedure to inject drug into an internal body tissue, thereby bypassing the epithelial barriers of the skin and gastrointestinal tract.

The primary therapeutic routes of drug administration are subcutaneous (SC or SQ) and intramuscular (IM). In these cases, the total dose of drug is known and injected into tissue that is well perfused by systemic capillaries that drain into the central venous circulation. Both of these routes as well as intravenous administration are termed *parenteral* to contrast primarily with oral (*enteral)* and topical dosing, which are classified as *nonparenteral* routes of drug administration. A primary difference between these two classes is that parenteral routes bypass all of the body's defensive mechanisms. Parenteral dosage forms are manufactured under strict guidelines that eliminate microbial and particulate contamination resulting in sterile preparations that must be administered using aseptic techniques. This restriction does apply to oral or topical dosage forms. As with all methods of drug administration, there are numerous variables associated with SC and IM dosing that can be conveniently classified into pharmaceutical and biological categories.

Finally, there are other occasional routes of drug administration employed that require absorption for activity. Administration of drugs by intraperitoneal injection is often used in toxicology studies in rodents since larger volumes can be administered. Peritoneal absorption is very efficient, provided adequate "mixing" of the injection with the peritoneal fluid is achieved. The majority of drug absorbed after interperitoneal administration enters the portal vein and thus may undergo first-pass hepatic metabolism. The disposition of intraperitoneal drug thus mirrors oral administration.

Some drugs are administered by conjunctival, intravaginal, or intramammary routes. In these cases achievement of effective systemic concentrations are often not required for what is an essentially local therapeutic effect. Prolonged absorption from these sites may result in persistent tissue residues in food-producing animals if the analytical sensitivity of the monitoring assay is sufficiently low. The systemic absorption of these dosage forms is quantitated using procedures identical to those employed for other routes of administration.

Bioavailability

The final topic to consider with absorption is the assessment of the extent and rate of absorption after oral, topical, or inhalational drug administration. The extent of drug absorption is defined as absolute systemic availability and is denoted in pharmacokinetic equations as the fraction of an applied dose absorbed into the body (F). Although this topic will also be discussed extensively in Chapter 3, it is important and convenient at this juncture to introduce the basic concepts so as to complete the discussion of drug absorption. If one is estimating the extent of drug absorption by measuring the resultant concentrations in either blood or excreta, one must have an estimate of how much drug normally would be found if the entire dose were absorbed. To estimate this, an intravenous dose is required since this is the only route of administration that guarantees that 100% of the dose is systemically available ($F = 1.0$) and the pattern of disposition and metabolism can be quantitated. Parameters used to measure systemic availability are thus calculated as a ratio relative to the intravenous dose.

For most therapeutic drug studies, systemic absorption is assessed by measuring blood concentrations. The amount of drug collected after administration by the route under study is divided by that collected after intravenous administration. When drug concentrations in blood (or serum or plasma) are assayed, total absorption is assessed by measuring the area under the concentration–time curve (AUC) using the trapezoidal method. This is a geometrical technique that breaks the AUC into corresponding trapezoids based on the number of samples assayed. The terminal area beyond the last data point (a triangle) is estimated and added together with the previous trapezoidal areas. Absolute systemic availability then is calculated as in Equation 2.4:

$$\mathrm{F}\,(\%) = \frac{\mathrm{AUC}_{\mathrm{route}} \quad \mathrm{Dose}_{\mathrm{iv}}}{\mathrm{AUC}_{\mathrm{iv}} \quad \mathrm{Dose}_{\mathrm{route}}} \tag{2.4}$$

Calculation of F provides only an estimate of the extent, and not rate, of drug absorption. To calculate rate, pharmacokinetic techniques are required and presented in Chapter 3. Finally, so-called relative systemic availability may be calculated for two nonintravenous formulations where the data for the reference product is in the denominator and the test formulation in the numerator.

Distribution

A toxicant absorbed into the systemic circulation following any route of administration must reach its site of action at a high enough concentration for a sufficient period of time to elicit a biological response. Distribution processes determine this outcome. There are numerous tissues to which a chemical may be distributed, some of them capable of eliciting a pharmacological or toxicological (intended versus unintended) response while others serve only as a sink or depot for the chemical. Sinks may also be formed as a result of chemical binding to tissue or plasma proteins. The toxicological significance of such sinks is that chemicals will be distributed to, and in some cases stored in, these tissues and only slowly released back into the systemic circulation for ultimate elimination. Such tissue binding may actually protect against acute adverse effects by providing an "inert" site for toxicant localization. Storage may, however, prolong the overall residence time of a compound in the body and promote accumulation during chronic exposure, two processes that would potentiate chronic toxicity.

If the animal is a food-producing species, such tissue storage may result in residues in the edible meat products. Tissue concentrations thus become an endpoint in themselves, devoid of a biological or toxicological relevance in the tissue they are found. Their relevance is set by regulations that legally establish safe tissue tolerances or maximum residue levels for specific tissues and species. These are based upon extrapolations of safety to the consuming human population and food consumption patterns. These concepts are developed in Chapter 61.

Distribution of chemicals to peripheral tissues is dependent on four factors:

1) Physiochemical properties of the compound (pKa, lipid solubility, molecular weight)

2) Concentration gradient established between the blood and tissue
3) Ratio of blood flow to tissue mass
4) Affinity of the chemical for tissue constituents.

The physiochemical properties of the chemical are most important in determining its propensity to distribute to a specific tissue. For most molecules, distribution out of the blood into tissue is via bulk flow through the capillary pores or by simple diffusion down a concentration gradient; hence distribution is generally described by first-order rate constants. One can conceptualize distribution as "absorption" into the tissues from the blood. The complicating factors are that the driving concentration is now dependent upon blood flow, the surface area for "absorption into tissues" is dependent upon capillary density and tissue mass, the relevant partition coefficient is the blood/tissue ratio, and plasma/tissue protein binding complicates the picture. An understanding of distribution is a prerequisite to predicting pharmacological response.

Physiological Determinants of Distribution

Body fluids are distributed between three primary compartments, only one of which, vascular fluid, is thought to have an important role in the distribution of most compounds throughout the body. Human plasma amounts to about 4% of the total body weight and 53% of the total blood volume. By comparison, the interstitial tissue fluids account for 13%, and intracellular fluids 41%, of body weight. Use of recently developed microdialysis and ultrafiltration probes and catheters allow the concentration of drug to be directly monitored in the interstitial fluid and thus further open the window for pharmacokinetic analysis. The concentration that a compound may achieve in the blood following exposure depends in part upon its apparent volume of distribution. If it is distributed only in the plasma, a high concentration could be achieved in the vascular system. In contrast, the concentration would be markedly lower if the same quantity of toxicant were distributed to a larger pool including the interstitial water and/or cellular fluids.

The next major consideration is the relative blood flow to different tissues. Two factors will potentiate chemical accumulation into a tissue: high blood flow per unit mass of tissue and a large tissue mass. Tissues with a *high blood flow/mass* ratio include the *brain, heart, liver, kidney,* and *endocrine glands*. Tissues with an *intermediate ratio* include *muscle* and *skin,* while tissues with a *low ratio* (indicative of poor systemic perfusion) include *adipose tissue* and *bone*. These ratios are generalizations and some tissues may actually be categorized in two disparate groups. An excellent example is the kidney where the renal cortex receives some 25% of cardiac output and thus has a very high blood flow/mass ratio. However, the renal medulla receives only a small fraction of this blood flow and thus could be categorized in the intermediate to low group. If the affinity of the chemical for the tissue is high, it will still accumulate in poorly perfused tissues (such as fat), although it will take a long period of time to "load" or "deplete" these tissues. A relatively low blood flow/mass ratio is a major physiological explanation for depot formation.

Tissue Barriers to Distribution

Some organs have unique anatomic barriers to xenobiotic penetration. The classic and most studied example is the blood–brain barrier, which has a glial cell layer interposed between the capillary endothelium and the nervous tissue (illustrated nicely in Figure 9.4). In the schematic membrane scheme depicted in Figure 2.2, this amounts to an additional lipid membrane between the capillary and target tissue. Only nonionized lipid-soluble compounds can penetrate this barrier. Similar considerations apply to ocular, prostatic, testicular, synovial, mammary gland, and placental drug or toxicant distribution. In addition, pH partitioning phenomenon also may occur since the protected tissue (e.g., cerebrospinal fluid) may have a lower pH than the circulating blood plasma. Chemicals may also distribute into transcellular fluid compartments, which are also demarcated by an epithelial cell layer. These include cerebrospinal, intraocular, synovial, pericardial, pleural, peritoneal, and cochlear perilymph fluid compartments.

A few tissues possess selective transport mechanisms that accumulate specific chemicals against concentration gradients. For example, the blood–brain barrier possesses glucose, L-amino acid, and transferrin transporters. If the toxicant resembles an endogenous transport substrate, it may preferentially concentrate in a particular tissue. Recent work with the blood–brain barrier has demonstrated that some of these tissues also possess drug efflux transport processes that remove drug from the protected sites. Two such processes are P-glycoprotein associated with multidrug resistance (MDR) and the weak organic acid cell-to-blood efflux systems.

P-glycoprotein is a member of the so-called ATP-binding cassette proteins that include the cystic fibrosis transmembrane regulator and the sulfonyurea-sensitive ATP-dependent potassium channel. Drugs such as vinblastine, vincristine, or cyclosporine, which have the proper physiochemical characteristics (high lipophilicity) to enter the brain, do not achieve effective concentrations because of this active efflux mechanisms. This transport system has recently been shown to cause the unique breed sensitivity of Collies to ivermectin toxicity. These transporters are also responsible for decreased bioavailability of some drugs due to active pumping of absorbed drug back into the intestinal lumen. A number of drugs also inhibit P-glycoprotein transport (e.g., ketoconazole, cyclosporine), which forms the basis for some complex drug–drug interactions. Similar processes and transport systems for peptides and other compounds are also found in other organs (e.g., liver). Chapter 50 in this text should be consulted for further details on P-glycoprotein.

Plasma Protein Binding

Following entry into the circulatory system, a chemical is distributed throughout the body and may accumulate at the site of toxic action, be transferred to a storage depot, or be transported to organs that will detoxify, activate, or eliminate the compound. Although many toxicants have sufficient solubility in the aqueous component of blood to account for simple solution as a means of distribution, the primary distribution mechanism for insoluble toxicants appears to be in association with plasma proteins. Although cellular components (e.g., red blood cells) may also be responsible for transport of drugs, such transport is seldom the major route. The transport of compounds by lymph is usually of little quantitative importance for many drugs, although it may be very important in delivering some lipophilic drugs and potentially nanoparticles to select organs. Both erythrocytes and lymph can play roles in the transport of some lipophilic drugs and toxins, in some instances to an important extent.

Studies of plasma proteins have shown albumin to be particularly important in the binding of drugs. This is especially true for weak acids, with weak bases often binding to acid glycoproteins. For certain hormones, specific high-affinity transport proteins are present. Studies of toxicant binding have been more limited, but there is evidence of a significant binding/partitioning role for lipoproteins in carrying very lipophilic chemicals in the blood. In the case of most drug–protein interactions, reversible binding is established, which follows the Law of Mass Action and provides a remarkably efficient means whereby drugs can be transported to various tissues. The strength of this association may be quantitated through the use of the dissociation constant, K_{diss}. Among a group of binding sites on proteins, those with the smallest K_{diss} value for a given drug will bind it most tightly. In contrast to reversible binding seen with most therapeutic drugs, agents like cisplatin and some potentially carcinogenic metabolites that are formed from chlorinated hydrocarbons (such as CCl_4) are covalently bound to tissue proteins. In this case, there is no true distribution of the drug as there is no opportunity for dissociation.

Once a molecule binds to a plasma protein, it moves throughout the circulation until it dissociates, usually for attachment to another large molecule. Dissociation occurs when the affinity for another biomolecule or tissue component is greater than that for the plasma protein to which the toxicant was originally bound. Thus, forces of association must be strong enough to establish an initial interaction, and they must also be weak enough such that a change in the physical or chemical environment can lead to dissociation. Dissociation could occur by binding to proteins of greater affinity (lower K_{diss} values), binding with a higher concentration of proteins of lower affinity, or changes in K_{diss} with changes in ionic strength, pH, temperature, or conformational changes in the binding site induced by binding of other molecules. As long as binding is reversible, redistribution will occur whenever the concentration of one pool (i.e., blood or tissue) is diminished. Redistribution must occur when the concentration is diminished in order to reestablish equilibrium.

Proteins complex with drugs by a variety of mechanisms. Covalent binding may have a profound direct effect on an organism due to modification of an essential molecule, but it usually accounts for a minor portion of the total dose and is of no importance in further distribution of drugs since such compounds cannot dissociate. As previously mentioned, when metabolites of some compounds are covalently bound to proteins, there may be no opportunity for subsequent release of the drug apart from release upon breakdown of the protein itself. The cancer chemotherapeutic drug cisplatin covalently binds to albumin through an aquation reaction. In incubation studies, "aging" occurs after a short period of time independent of drug concentration and the majority of circulating cisplatin is covalently bound.

Noncovalent binding is of primary importance with respect to drug distribution because of the opportunities to dissociate after transport. In rare cases, the noncovalent bond may be so tight (K_{diss} extremely small) that a compound remains in the blood for very lengthy periods. For example, 3-hydroxy-2,4,4-triiodo-α-ethyl hydrocinnamic acid has a half-life of about 1 year with respect to its binding to plasma albumin. The new cephalosporin antimicrobial cefovecin similarly is approximately 97% bound in dogs and has a half-life of 5.5 days, very long for this class of drugs.

Charged drugs may be bound to plasma proteins by ionic interactions. Electrostatic attraction occurs between two oppositely charged ions on a drug and a protein. Proteins are thereby capable of binding charged metal ions. The degree of binding varies with the chemical nature of each compound and the net charge. Dissociation of ionic bonds usually occurs readily, but some members of the transition group of metals exhibit high association constants (low K_{diss} values) and exchange is slow. Ionic interactions may also contribute to binding of alkaloids with ionizable nitrogenous groups and other ionizable toxicants. Hydrogen bonds arise when a hydrogen atom, covalently bound to one electronegative atom, is "shared" to a significant degree with a second electronegative atom. As a rule, only the most electronegative atoms (O, N, and E) form stable hydrogen bonds. Protein side chains containing hydroxyl, amino, carboxyl, imidazole, and carbamyl groups can form hydrogen bonds, as can the N and O atoms of peptide bonds themselves. Hydrogen bonding plays an important role in the structural configuration of proteins and nucleic acids. Van der Waals forces produce weak interactions, which act between the nucleus of one atom and the electrons of another atom, i.e., between dipoles and induced dipoles. The attractive forces arise from slight distortions induced in the electron clouds surrounding each nucleus as two atoms are brought close together. The binding force is critically dependent upon the proximity of interacting atoms and diminishes rapidly with distance. However, when these forces are summed over a large number of interacting atoms that "fit" together spatially, they can play a significant role in determining specificity of toxicant–protein interactions. A final mechanism of binding is based on hydrophobic interactions. When two nonpolar groups come together, they exclude the water between them, and this mutual repulsion of water results in a hydrophobic interaction. The minimization of thermodynamically unfavorable contact of a polar grouping with water molecules provides the major stabilizing effect in hydrophobic interactions.

Methods for Assessing Protein Binding

A number of methods have been employed to study drug–protein interactions, including ultrafiltration, electrophoresis, equilibrium dialysis, solvent extraction, solvent partition, ultracentrifugation, spectrophotometry, and gel filtration. The most widely used techniques are ultrafiltration and equilibrium dialysis. The basic concept is that a semipermeable membrane is used, which restricts passage of protein but allows unbound drug to cross the barrier according the diffusion. Bound drug is placed on one side of the membrane and samples are collected from the protein-free side. Ultrafiltration allows rapid protein–drug separation while equilibrium dialysis requires time for the separation to occur. The fraction of free drug is then calculated based on the amount of total drug used.

Protein-binding data are frequently expressed in terms of percent of drug bound. Although useful, the limitations should be recognized, for as drug concentration is lowered, the percentage of binding increases. When a compound has a high affinity for a protein (e.g., albumin), percent binding falls sharply when the total drug concentration exceeds a certain value that saturates the binding sites available.

Displacement

If a toxicant or drug is administered after binding sites on a protein are occupied by another chemical, competition for the site occurs, and a higher concentration of free drug may be available. Competition for the same site on plasma proteins may have especially important consequences when one of the potentially toxic ligands has a very high affinity. If compound A has low fractional binding (for example, 30%) and compound B displaces 10% of A from the protein, the net increase of free A is from 70% to 73%, a negligible increase. However, if A were 98% bound and 10% is displaced, the amount of free A increases from 2% to 12%, a sixfold increase in free toxicant. A change in binding may also occur when a second drug produces an allosteric effect resulting in altered affinity of the protein for the originally bound drug (noncompetitive binding). There is great debate as to the clinical significance of such drug–protein displacements that increase drug free fraction, since, as will be seen in the next section on mechanisms of free-drug elimination, the increased free concentration of drug may result in its increased elimination from the body, negating any enhanced activity or toxicity secondary to the displacement.

Most pharmacokinetic models in both human and comparative medical literature assess only total drug concentrations. When the extent of protein binding differs dramatically between species, inappropriate extrapolations often occur if the drug is very highly protein bound in one species. Similarly, interpretation of the extent of tissue distribution when the extent of protein binding is not known may be misleading. The most precise predictions can often be made when the free fraction of drug is known over the concentration ranges of the study being conducted.

Other Factors Affecting Distribution

Among the factors that affect distribution, apart from binding to blood macromolecules per se, are the route of administration, molecular weight, rate of metabolism, polarity, and stereochemistry of the parent compound or metabolic products, and rate of excretion. Molecular weight, charge, and/or polarity have been previously discussed. Stereoselectivity in the disposition of a drug is an often ignored phenomenon which could influence many studies. Its impact on metabolism is obvious; however, any receptor-mediated binding or transport process, including high-specificity protein binding could be affected. Propranolol and ibuprofen have been shown to demonstrate stereoselective distribution.

A major factor determining distribution is the extent of tissue binding, a process identical to that of serum protein binding except that the results on drug disposition are opposite. Tissue binding is governed by the same mechanisms as discussed above and tends to increase a drug's distribution, although not necessarily activity, if the drug is sequestered away from active drug receptors or target microorganisms. Covalent binding also occurs and is relevant to toxicology and tissue residue depletion. Depending on the pharmacokinetic model employed, irreversible covalent tissue binding may actually be mathematically detected as an increase in the drug's elimination, if only blood samples are used in the analysis, since there is no redistribution of drug back into the blood. For distribution to be quantitated, the basic assumption in most pharmacokinetic models is that the process is irreversible and thus ultimately an equilibrium will be achieved between drug movement into and out of tissue. When irreversible binding occurs, compound is extracted from blood and when excretory output (e.g., urine, feces, expired air) is not monitored, this is interpreted in many models as elimination. These model assumptions are often ignored.

As can be appreciated from this discussion, there are numerous factors that could affect distribution of a compound to tissues. Another is the methodology used to assess tissue distribution. Autoradiography is an excellent technique to anatomically localize distributed drug to the level of organs, cells, and even subcellular components. However, most pharmacokinetic studies rely on analytical techniques. When a tissue sample is collected from an animal, the sample is actually a homogenate of cells, extracellular fluid, and blood. The concentration measured cannot be uniquely assigned to any specific tissue or body fluid compartment. The use of microdialysis and ultrafiltration provides a direct estimate of extracellular fluid concentrations.

The extent of distribution of a compound is termed its volume of distribution (*Vd*) and is calculated by equations such as Equation 2.5:

$$\mathrm{Vd}(1) = \mathrm{Dose\ (mg)/Concentration\ (mg/l)} \qquad (2.5)$$

The *Vd* is actually a proportionality constant relating the plasma concentration to administered dose. Actual approaches to determine this will be presented in Chapter 3.

Renal Elimination

The ultimate route for drug elimination from the body is the kidney. Drugs can also be eliminated in bile, sweat, saliva, tears, milk, and expired air; however, for most therapeutic drugs these routes are generally not quantitatively important as mechanisms for reducing total body burden of drug. The degree of lipid solubility and extent of ionization in blood determines how much of drug will be excreted by the kidney. For drugs that are first biotransformed by the liver, the more water-soluble metabolites are then ultimately excreted through the kidney into the urine. The kidney has also been the most widely studied excretory organ because of the accessibility of urine to collection and analysis. Many of the principles utilized by pharmacologists in quantitating excretory organ function, especially clearance, were originally developed by renal physiologists to noninvasively assess kidney function. Dr. Homer Smith's classic reference on renal physiology is still instructive for the determination of renal clearance.

There are two components relevant to any discussion of renal drug excretion: physiology and quantitation. Renal drug excretion can be considered using the same principles of membrane transport developed earlier, except in this case the movement is from the vascular system to outside the body. Generally, only drugs that are either dissolved in the plasma or bound to circulating blood proteins are available for excretion. The pharmacokinetic parameter estimated by most of these approaches is the renal clearance of the drug.

Renal Physiology Relevant to Clearance of Drugs

For a perspective of drug excretion from the body, the kidney will be considered only as an excretory organ designed to remove foreign compounds (e.g., drugs) and metabolic by-products (e.g., creatinine, urea) from the blood. As will become evident, the major clinical indices of renal function such as blood urea nitrogen, serum creatinine, and creatinine clearance are actually pharmacokinetic parameters of creatinine and urea excretion!

The kidney receives approximately 25% of the cardiac output and thus processes a prodigious amount of blood. The kidney functions in a two-step manner to accomplish its function. The first step is passage through a filtering unit to retain formed cellular elements (e.g., erythrocytes, white blood cells) and proteins in the blood, only allowing the passage of plasma fluid into the remainder of the kidney. The second step utilizes a system of anatomically and physiologically segmented tubules to further modify the contents of the filtered fluid depending on a host of physiological needs including but not limited to fluid, electrolyte, and acid–base balance and the regulation of systemic blood pressure.

The primary functional unit of the kidney is the nephron depicted in Figure 2.8. Depending on the species, there may be 500,000 nephrons per kidney. The sum of their individual function is the observed organ function. Their specific anatomical arrangement is species dependent, often determined by the evolutionary adaptation of the animal to its environment relative to the need to conserve body fluids. The filtration unit is the glomerulus, while the remainder of the fluid processing is accomplished by the extensive tubular system, whose segments are named in relation to their relative distance

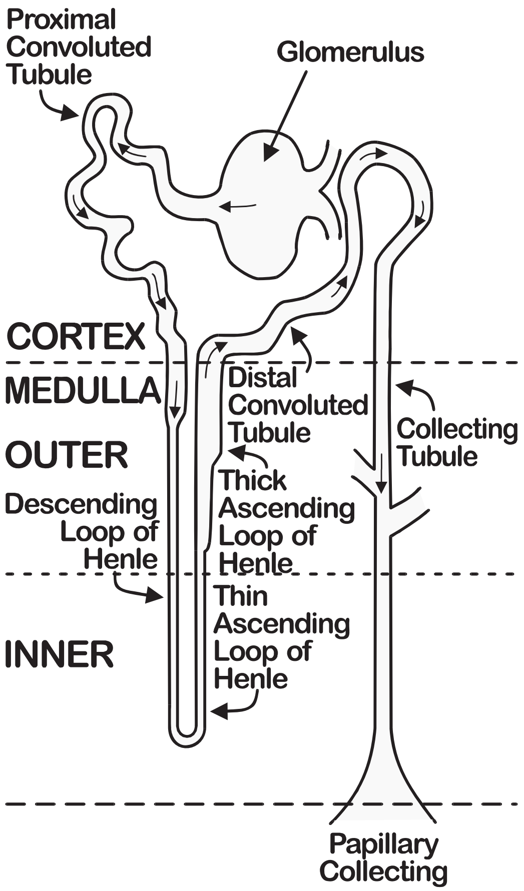


Figure 2.8 Structure of a nephron.

(proximal versus distal) measured *through* the tubules from the glomerulus. The junction between these is a unique anatomical adaptation called the loop of Henle that is designed to use countercurrent exchangers to efficiently produce a concentrated urine since most of the water that is filtered by the glomerulus must be reabsorbed back into the body. The loop of Henle also forces the distal tubules to return toward the surface of the kidney to interact with the glomeruli. Grossly, the region of the kidney containing the glomeruli as well as the proximal and returning distal tubules are on the outside toward the surface and comprise the renal cortex. This region of the kidney is very well perfused by blood and is primarily characterized by oxidative metabolic processes. The interior region is the medulla that is occupied by the penetrating loops of Henle, is poorly perfused, and is characterized by anaerobic metabolism. The amount of tubular fluid filtered by the glomeruli is acted upon by the various nephron segments to reabsorb wanted materials (primarily water and sodium) back into the blood and to let the remainder be excreted into the urine.

The kidney is also the site where acid–base balance is metabolically tuned by controlling acid and base excretion. Some of these processes are coupled to electrolyte secretion (e.g., potassium and sodium) and thus are further modulated by hormones such as aldosterone. These nephron functions may inadvertently alter the amount of drug eliminated in the tubules by changing tubular fluid pH and consequently the ionized fraction of weak acids and bases according to the Henderson–Hasselbach equation presented earlier. This modification in tubular fluid may affect the value of renal clearance determined in pharmacokinetic studies.

There are specific tubular transport systems that excrete products directly into the tubular fluid, which are not filterable because of plasma protein binding. Other transport systems reabsorb essential nutrients (e.g., glucose) back into the blood that were filtered into the tubular fluid. Drugs are also processed by these same transport systems making drug excretion dependent upon the physiological status of the animal. This is especially true when a drug biochemically resembles an endogenous substrate. As is similar to all transport processes, saturation and competition may occur, which may impart nonlinear behavior on a drug's kinetics.

Mechanisms of Renal Drug Excretion

Drugs are normally excreted by the kidney through the processes of (i) glomerular filtration, (ii) active tubular secretion and/or reabsorption, and/or (iii) passive, flow-dependent, nonionic back diffusion. These processes can be considered as vectorial quantities, each possessing magnitude and direction relative to transport between tubular fluid and blood. Their sum determines the ultimate elimination of a specific drug by the kidney as illustrated in Figure 2.9. *The total renal excretion of a drug equals its rate of filtration plus secretion minus reabsorption.* If a drug is reabsorbed back from the tubular fluid

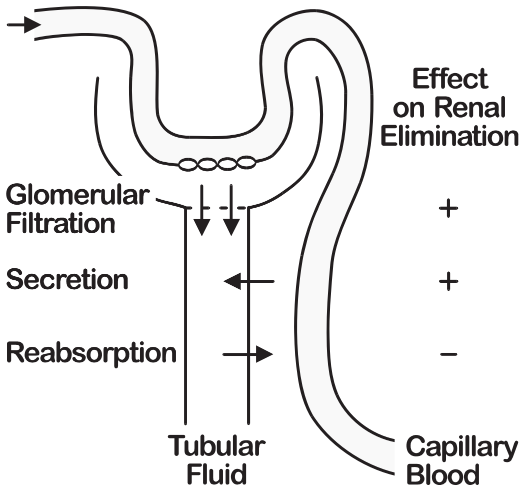


Figure 2.9 Vectorial processes of nephron function and their net effect on overall renal drug elimination.

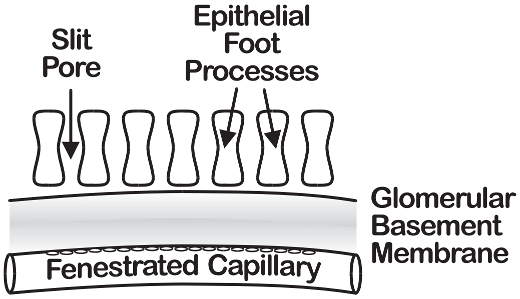


Figure 2.10 The glomerular filtration barrier.

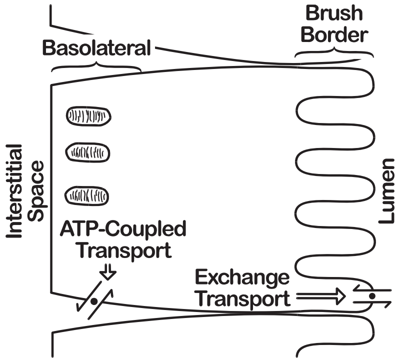


Figure 2.11 Schematic of a renal tubular cell illustrating location of active and exchange transport systems.

into the blood, its net renal excretion will be reduced. In contrast, if a drug is secreted from the blood into the tubular fluid, its net excretion will be increased. These events will be subsequently quantitated.

Excretion by glomerular filtration is unidirectional with drug removal from the blood by bulk flow. Only non-protein-bound drugs are eliminated by this process, a characteristic that is important when predicting elimination pathways for drugs. The rate of drug filtration, therefore, is dependent upon both the extent of drug protein binding and the glomerular filtration rate (GFR) whose calculation will be developed below.

Glomerular filtration is essentially ultrafiltration through the relatively permeable glomerular filtration barrier, which consists of the epithelial cells of Bowman's capsule, the glomerular basement membrane, and the slit-pores formed from juxtaposing epithelial foot processes (Figure 2.10). These possess a fixed negative charge that is a major contributor to the rate-limiting aspect of this barrier. When damaged, filtration selectivity is impaired and proteins may pass into tubular fluid. This is the primary manifestation of glomerular diseases that affect drug excretion.

The magnitude of active tubular secretion is not affected by the extent of plasma drug protein binding. These saturable, carrier-mediated processes are energy dependent and described by the laws of Michaelis–Menten enzyme kinetics. In order to promote absorption from the tubular filtrate into blood, tubule cells have microvilli, much like the intestinal mucosal cells, which maximize the surface area to cell volume ratio presented to the tubule. For secretion from the interstitial space into the tubule lumen, the basolateral surfaces of these cells (side facing the capillaries) have intensive membrane invaginations that also increase the surface area for interaction with the perfusing capillaries to facilitate active secretion. To provide the energy to drive these processes, proximal tubule cells have high mitochondrial densities to generate ATP, which fuels the Na^+-K^+ ATPase-coupled transport systems. This high level of oxidative metabolism is the primary reason for the sensitivity of the kidney to hypoxic or anoxic conditions, which result in renal damage if blood perfusion is interrupted even for short periods of time.

The cellular structure of transport systems across tubule cells involves two separate pairs of transport proteins, which creates an overall "polarity" of tubule cell function relative to the interstitial fluid and tubular lumen (Figure 2.11). One set is located in the brush border of the interface with tubular fluid and the other is located in the basolateral membrane. Energy coupling with ATP generally occurs in the basal portion of the cell (proximity to mitochondria), which, in secretion, builds up intracellular drug concentrations that are then transported to the tubular fluid by concentration-driven facilitated transport carriers. In reabsorption, the reverse occurs as the basolateral active "pumps" create low intracellular drug concentrations, which promote facilitated carrier-mediated reabsorption through the brush-border tubular membrane. Most transport systems are also stoichiometrically coupled to the transport of an electrolyte (e.g., Na^+, K^+, Cl^-, H^+), which assures electrical neutrality and provides a mechanism for modulating the systemic concentrations of these elements. The primary ion, which drives these transporters and which regulates overall renal function, is sodium. Thus, all drug transport systems are usually coupled to a Na^+ ATPase transmembrane system whose structure and polarity will determine the nature and direction of drug movement.

There are two distinct secretory pathways in the later sections of the proximal renal tubule that are relevant to a discussion of drug and toxicant excretion: one for acidic and one for basic compounds; the so-called organic anion and organic cation transporters. There are multiple types within each class and most do not show absolute substrate specificity. The primary orientation of this system is from blood to tubular filtrate, removing drugs and/or metabolite conjugates from the blood. Active reabsorption systems are also present that act on a drug already present in the filtered load. These systems are generally present to recover essential nutrients (e.g., glucose) that have been filtered by the glomerulus. Some drugs reach their target sites by this mechanism making their tubular fluid concentration more important for predicting activity than their blood concentrations. An excellent example is the diuretic furosemide that is first secreted by the tubules into the tubular fluid and then is actively reabsorbed back into the tubular cells to gain access to its receptors for activity. Thus, the best concentration–time profile to predict the diuretic action of furosemide is that of the urine rather than blood.

Drugs (and other endogenous substrates) may compete for tubular transport sites, thereby functioning as reversible, competitive inhibitors. This interaction has been classically studied with the organic acid transport system. Weak acids such as probenecid or phenylbutazone will inhibit secretion of the weak acid penicillin, thereby prolonging penicillin blood concentrations. Thus, when more than one drug in the same ionic class is administered, and they compete for the same organic ion transporter, their rate and extent of renal excretion will be affected. Many drug metabolites are conjugates (e.g., glucuronides) produced by Phase II hepatic biotransformation reactions and secreted by the transport system for weak acids, which may further complicate the pattern of drug excretion.

There are direct pharmacokinetic implications to the carrier-mediated mechanism of renal tubular drug secretion. The limited capacity of carrier-mediated processes means that above certain blood drug concentrations, transport will proceed at a maximal rate independent of concentration in blood; that is, so-called *nonlinear zero order kinetics* will become controlling, which will have adverse effects on the utility of normal linear pharmacokinetic models. These factors may become more important in renal disease states where renal capacity is already diminished. Under these circumstances, drug renal clearance will approach the glomerular filtration rate because additional drug concentrations in blood will not now be secreted into the urine. At subsaturation concentrations, renal clearance of an actively secreted substance is dependent on and limited by renal plasma flow and thus flow-limited mechanisms discussed below will become important considerations.

The final determinant of a drug's renal disposition is the mechanism of nonionic passive tubular reabsorption, or back diffusion, a process dependent upon urine flow rate, lipid solubility of the nonionized drug moiety, and urine pH. At low urine flow rates, there is greater opportunity for diffusion of drug from the distal tubular fluid back into the blood. Diffusion is facilitated by the high concentration of drug in the tubular fluid. Polar compounds having low lipid solubility, such as many drug metabolites, are not reabsorbed since they cannot cross the lipid membrane. In contrast, lipid-soluble, nonionized drugs are reabsorbed into the blood. The ratio of ionized to nonionized molecules determines the concentration gradient that drives the drug into the fluid. The extent of reabsorption is again a function of the drug's pKa and the pH of the tubular fluid, as described by the Henderson–Hasselbach equations (see Equations 2.2 and 2.3). The pH of the urine can undergo drastic changes as a function of diet and coadministered drugs (e.g., urine acidifiers and alkalizers). Tubular reabsorption of organic acids occurs with pKa values between 3.0 and 7.5 and for basic drugs with pKa values between 7.5 and 10.5. Weak acids thus are reabsorbed at low urinary pH (acidic), while weak bases are reabsorbed at high urinary pH (alkaline). Therefore, the renal excretion of an acidic drug decreases in acidic urine but increases in alkaline urine.

This principle is employed in treating salicylate intoxication in dogs. A brisk, alkaline diuresis is induced to decrease salicylate reabsorption into the blood and hasten excretion into the urine by trapping the salicylic acid in an ionized form in the alkaline urine. Reabsorption is further decreased by the elevated urinary flow rate. In contrast, induction of an alkaline diuresis will enhance the toxicity of basic drugs by increasing the amount of tubular reabsorption. Drugs often employed in critical care situations, such as procainamide or quinidine, have increased reabsorption and thus systemic activity in this alkaline state.

Species differences in urinary pH can have a major influence on the rate of renal excretion of ionizable drugs. Carnivores tend to have a more acidic (pH 5.5–7.0) urine than herbivores (pH 7.0–8.0). Thus, with all other disposition factors being equal, a weakly acidic drug will have a higher renal excretion in herbivores than in carnivores and a weakly basic drug will have a greater renal excretion in carnivores than in herbivores. In healthy animals, small changes in urinary pH or urine flow rate do not significantly contribute to altered drug clearance. However, with decreased function in renal disease, there is a decreased tubular load of drug. Altered urinary pH theoretically could further decrease overall drug clearance.

There are two other peculiarities of renal tubular transport that must be discussed before quantitating these processes. Some drugs are reabsorbed into the tubules by pinocytosis. This occurs by interaction of filtered drug in the tubular fluid with the brush border membrane. This is a very low-capacity and slow process that is easily saturated. Pinocytozed drugs are then transferred to lysosomes and generally digested in the cell (e.g., peptides and filtered proteins such as β_2 microglobulin). However, for some compounds, such as the aminoglycosides, enzymatic breakdown does not occur and the drug is essentially stored in the kidney. Therefore, although the drug is reabsorbed from the tubular fluid, it is not transported through the cell into the blood. Thus unlike other tubular reabsorption processes, reabsorption with storage or metabolism does decrease elimination of drug from the body. Such reabsorption has toxicological significance because the drug does accumulate in the tubular cells and could produce an adverse effect. Finally, this phenomenon has a major influence on the prediction of tissue residue profiles in the kidney resulting from drugs with prolonged elimination half-lives (e.g., aminoglycosides).

The final confounding influence on determination of renal drug clearance is when a drug is metabolized by the kidney. Most of the Phase I and Phase II enzymatic systems present in the liver also exist in the kidney, although different isozymes may be expressed. Oxidative processes generally occur within the proximal tubule cells. Two scenarios may occur. The first is when the drug is solely metabolized by the kidney and not the liver, or a combination of both processes occurs. The second is when *relay* metabolism occurs and the kidney further metabolizes a drug already biotransformed by the liver. These interactions are complex and generally are of toxicological significance.

Renal drug biotransformation may also occur in the medulla by anaerobic metabolic processes (e.g., prostaglandin endoperoxide synthetase). This process is small relative to reducing overall body burden because only 1% of renal blood flow delivers compounds to this region, but it has toxicological significance to the renal medulla where drug and/or metabolite may accumulate. Finally, brush border enzymes are present that metabolize peptides in the filtered tubular load to amino acids for reabsorption. Stereoselectivity in both active tubular secretion and metabolism in the kidney may occur with specific drugs (e.g., quinidine). The implications to assessment of renal drug excretion is similar to that of drugs metabolized by the kidney and is usually not taken into account.

The Concept of Clearance and its Calculation

Clearance is a concept widely used to measure the efficiency of drug elimination from an organ or the whole body. The concept was developed for use in assessing kidney function by renal physiologists. The problem with simply measuring the concentration of drug in urine as an index of its renal excretion is that the kidney also modulates the volume of urine produced in association with its primary mission of regulating fluid balance. Thus, the concentration of drug alone may be higher or lower depending on the ultimate urine volume. To accurately assess how much drug is eliminated, the product of volume of urine produced and the concentration of drug in urine (mass/volume) must be determined to provide the amount excreted (mass). If timed urine samples are collected, an excretion rate (mass/time) is determined. Similarly, to assess how efficient this process is, one must know how much drug is actually presented to the kidney for excretion. This is related to the concentration of drug in the arterial blood. The physiological concept of clearance was developed by early workers to generate a parameter that measured the true efficiency of renal excretion processes by assessing the total mass of compound ultimately excreted and relating it to the concentration of drug presented to the kidney for excretion.

There are two definitions of renal clearance that are used to define equations to calculate this parameter from real data. The first is *the volume of blood cleared of a substance by the kidney per unit of time*, that is, the volume of blood required to contain the quantity of drug removed by the kidney during a specific time interval. This will be derived when we have developed pharmacokinetic parameters in Chapter 3 to quantitate Vd and fractional excretion rates. The second definition is *the rate of drug excretion relative to its plasma concentration*. In both cases, the actual value for a drug's renal clearance is the vectorial sum of {filtration + tubular secretion − tubular reabsorption}, making it a parameter that estimates the entire contribution of the kidney to drug elimination. Similarly, any change in renal drug processing will be reflected in renal clearance *if* it is not compensated for by more distal components of the renal tubules.

There are two types of data needed to calculate clearance: (i) an estimate of blood drug concentration presented to the kidney and (ii) the amount of drug removed by the kidney. The latter can be estimated either by measuring the amount of drug excreted by the urine or comparing the difference between the renal arterial and venous drug concentrations to assess how much drug was extracted while passing through the organ.

To begin, we will use the classic approach (Equation 2.6), which directly measures extraction, based on Fick's Law:

$$\mathrm{Cl\ (ml/min) = (Q)\ (E) = (Q)\ \frac{\left(C_{art} - C_{ven}\right)}{\left(C_{art}\right)}} \tag{2.6}$$

where Q is renal arterial blood flow (ml/min), E is the extraction ratio and C_{art} and C_{ven} are arterial and venous blood concentrations. The obvious difficulty with this approach is that arterial and venous blood samples must be collected. However, renal physiologists realized that this approach could be modified to more easily assess renal function if a few assumptions were made.

The first is that the amount of substance removed or extracted by the kidney is equivalent to that which is excreted into the urine. If one makes a timed collection of urine and measures the urine concentration and volume, the amount (X) of drug extracted by the kidney over a specific time interval, that is, its *rate* of renal excretion denoted $\Delta X/\Delta t$, is the following (Equation 2.7):

$$\mathrm{\Delta X/\Delta t\,(mg/min) = \left[U_X\,(mg/ml)\right]\,[V\,(ml/min)]} \tag{2.7}$$

where U_x is the concentration of drug in urine and V is the urine production. Now the only component needed is the concentration presented to the kidney. Workers used constant rate intravenous infusion of chemicals to insure that so-called *steady state* blood concentrations were achieved. With this experimental design, the renal clearance of substance X is calculated as the following (Equation 2.8):

$$\mathrm{Cl}_{(\mathrm{renal})}\ (\mathrm{ml/min}) = (\Delta X/\Delta t)/C_{\mathrm{art}} = \mathbf{U_X}V/C_{\mathrm{art}} \quad (2.8)$$

This expression is equivalent to the definition for clearance above that relates the rate of drug excretion to its plasma concentration. This expression serves as the basis for many of the pharmacokinetic techniques to be developed in subsequent chapters.

Some minor discrepancies may result when drug clearances are calculated by use of blood or plasma data alone versus techniques such as those that employ urine collection. As discussed earlier, tubular reabsorption with storage (e.g., aminoglycoside antibiotics) will result in a lower $\mathrm{Cl}_{(\mathrm{renal})}$ calculated from urine data rather than blood-based methods because tubular reabsorption would not be reflected in the venous blood concentrations since the substance is now trapped in the tubular cells. A similar discrepancy may occur with intrarenal drug metabolism since this process does not return parent drug to venous blood. In a research setting, the difference is often used as conclusive evidence that either of these two phenomena actually occur.

Nonlinearity of Tubular Secretion and Reabsorption

The discussion thus far has been limited to determining clearances of substances that are primarily eliminated through glomerular filtration. The pharmacokinetics of this process are linear since saturation does not occur and only non-protein-bound drugs are filtered through the glomerular basement membrane complex. When the renal clearance of a drug eliminated by glomerular filtration is estimated, only the filtration of the free or unbound drug is assessed; thus changes in protein binding will change the net excretion of the drug. Drugs and toxicants that undergo passive tubular reabsorption obey Fick's Law of Diffusion since concentration gradients described again by linear first-order rate constants provide the driving force across the tubular epithelium. In contrast, compounds that are actively secreted from postglomerular capillaries across the renal tubules and into the tubular fluid show saturation at high concentrations, competition with drugs secreted by the same pathways, and dependence on the magnitude of renal blood flow; all hallmarks of nonlinear pharmacokinetic behavior. For such compounds, clearance will not be constant but rather will be dependent upon the concentration of drug presented to the kidney.

As tubular secretory pathways become saturated, a drug's clearance will decrease. To develop this concept, we will revisit our definition of a drug cleared by GFR and acknowledge that only the free or unbound drug concentration (C_f) is eliminated by filtration. Protein bound drug (C_b) cannot be filtered. Therefore, the rate of renal excretion ($\Delta X/\Delta t$) can be expressed as simply this (Equation 2.9):

$$\Delta X/\Delta t = C_f \times \mathrm{GFR} \quad (2.9)$$

As C_f becomes greater, $\Delta X/\Delta t$ will increase in direct proportion (e.g., linearly increase). However recalling Equation 2.8, its clearance will be $\Delta X/\Delta t$ *divided* by C_{art}. In this case, C_{art} is the total blood concentration presented to the kidney ($C_f + C_b$). Clearance thus equals the following (Equation 2.10):

$$\mathrm{Cl}_{(\mathrm{renal})} = (\Delta X/\Delta t)/C_{\mathrm{art}} \quad (2.10)$$

These relations have two implications. The first is that as total blood concentrations of drug increase, so does $\Delta X/\Delta t$; however, $\mathrm{Cl}_{(\mathrm{renal})}$ remains constant (Equation 2.11) because

$$\mathrm{Cl}_{(\mathrm{renal})} = (C_f \times \mathrm{GFR})/(C_{\mathrm{art}}) \quad (2.11)$$

and C_{art} will increase in direct proportion to $C_f + C_b$ as long as the fraction bound does not change. However, if the extent of protein binding of a drug is increased ($C_f \downarrow$, $C_b \uparrow$), its rate of renal excretion, $\Delta X/\Delta t$, will decrease as will its clearance since the C_{art} ($C_f + C_b$) will be constant. Therefore, drugs cleared by filtration have constant clearance with changing total drug concentrations but are sensitive to the extent of protein binding. This is one reason mentioned above in the discussion of plasma protein binding, that displacement of a protein bound drug that increases C_f will result in increased renal clearance, which then reduces its concentration to normal. For such a drug with high protein binding, only the small fraction presented for filtration can ever be extracted and cleared by the kidney. Since the total renal clearance of a compound is the sum of filtration plus secretion, a drug *solely cleared by filtration* will have a relatively low clearance compared to one that is also actively secreted. If one considers this in terms of the extraction ratio (E) defined above, Cl_B will always be less than the renal blood flow (Q) since the extraction ratio is less than one and dependent on the glomerular filtration fraction. Such drugs are termed *low extraction* drugs and their $\mathrm{Cl}_{(\mathrm{renal})}$ will be sensitive to the extent of protein binding. Examples of such drugs include inulin, the aminoglycoside antibiotics, tetracyclines, digoxin, and furosemide.

In contrast, consider a drug that also undergoes active tubular secretion. In this case, even a drug that is protein bound (C_b) or distributed into red blood cells will be secreted into the urine since the affinity for specific tubular transport proteins will be greater than that for the relatively nonspecific protein-binding sites or partitioning in erythrocytes. The extraction ratio will thus approach 1.0 and $Cl_{(renal)}$ will approach the renal blood flow Q. Such drugs are termed *high extraction* or *perfusion limited* to acknowledge the relationship of clearance to blood flow. The classic example is para-amino hippurate (PAH) because it is almost completely extracted as it passes through the kidney, making its clearance almost equal to renal plasma flow. In fact, PAH renal clearance had once been used in clinical situations to estimate renal blood flow. Other such drugs include many of the β-lactam antibiotics (e.g., penicillin) and many sulfate and glucuronide conjugate products of hepatic drug biotransformation. Another implication of active tubular secretion is that at sufficiently high concentrations, saturation of the secretory pathways may occur. Finally, the maximal renal clearance possible is renal blood flow.

The final pathway modulating renal excretion is when a drug undergoes passive tubular reabsorption. The dependency of this process on urinary pH has already been discussed. In this case, C_{art} will be constant but $\Delta X/\Delta t$ and thus $Cl_{(renal)}$ will vary depending on the urinary pH. Since this is an equilibrium process, time is required for this diffusion to occur. Thus if the renal clearance of a drug is dependent on urine flow, it is presumed to undergo passive tubular reabsorption. When high tubular loads are presented, reabsorption is overloaded as equilibrium cannot be achieved and nonreabsorbed drug is eliminated into the urine.

We have focused this discussion on renal drug elimination. However, clearance is used throughout physiology and pharmacokinetics to quantitate drug elimination through any organ as well as from the body. The relevant equation (Equation 2.12) defining the whole body clearance (Cl_B) of a drug is the sum of all elimination clearances:

$$Cl_B = Cl_{(renal)} + Cl_{(hepatic)} + Cl_{(other)} \tag{2.12}$$

Calculation of Cl_B provides an efficient strategy for estimating how a drug or toxicant is eliminated from the body as it indirectly compares systemic clearance to renal and hepatic clearances.

Hepatic Biotransformation and Biliary Excretion

Hepatic disposition is one of the final keys in the ADME scheme needed to describe the disposition of many drugs and chemicals in the body. The liver is responsible for both biotransformation and biliary excretion. In many ways, the liver should be considered as two separate organs, one encompassing metabolism and the other biliary excretion.

Drug localization and biotransformation in the liver are dependent on many factors associated with both the biological system and drug itself. These factors include the biological properties of the liver (chemical composition, relative activity of major drug metabolism enzymes, hepatic volume/perfusion rate, and drug accessibility to and extraction by hepatic metabolic sites) as well as the physicochemical properties of the drug (pKa, lipid solubility, molecular weight). In a quantitative sense, the liver is the major drug metabolism organ in the body.

Species differences in drug metabolic fate are, in most cases, the primary source of variation in drug disposition and, therefore, in drug activity or toxicity, across species. It has been estimated that 90% of drugs administered to humans are metabolized and polymorphisms in human metabolizing enzymes are responsible for the vast majority of adverse drug events. Extrapolation of metabolism data between animal species is an important issue as is the ability to correlate in vivo pharmacokinetic and metabolic data with in vitro metabolic findings.

Recalling our earlier discussion about the phenomenological role of metabolism in drug distribution and excretion, it would be hard to imagine what would happen in biological systems without xenobiotic metabolism. Absorbed compounds would stay in the body for a much longer period of time and have prolonged activity, tissue accumulation, and, potentially, toxicity. Metabolism is necessary for the animal or human body to rid itself of lipophilic xenobiotics as an effective defense mechanism against adverse effects. In general, the intensity of drug action is proportional to the concentration of the drug and/or its active metabolite(s) at the target site. On the other hand, drug-associated toxicity is also dependent on the chemical form (active or inactive) and concentration at the same or other relevant target site. Therefore, any process or factor that modifies the drug/metabolite concentration at a target site will cause an altered activity or toxicity profile. Drug metabolism may often result in metabolite(s) with altered chemical structures, which change the receptor type affected, drug–receptor affinity, or pharmacological effect. Most parent drugs can be deactivated to inactive metabolites. In contrast, some drugs can also be activated either from an inactive form (prodrug) to an active drug, or from an active form (e.g., meperidine) to an active metabolite (normeperidine) with similar activity/toxicity. Therefore, drug metabolism can either reduce or enhance parent drug's effect, create another activity, or even elicit toxicity, depending on both the drug and the biological system in question.

Therefore, the pharmacological and pharmacokinetic properties of a drug can be changed by metabolism in one or several of the following ways: pharmacological

Table 2.2 Drug metabolism reactions

Phase I	Phase II
Oxidation	Glucuronidation/glucosidation
Cyt P-450 dependent	Sulfation
Others	Methylation
Reduction	Acetylation
Hydrolysis	Amino acid conjugation
Hydration	Glutathione conjugation
Dethioacetylation	Fatty acid conjugation
Isomerization	

activation or deactivation; change in disposition kinetics of drug uptake (absorption from application site), distribution, and excretion (e.g., bile excretion, enterohepatic circulation, and renal excretion). The remainder of this chapter focuses on hepatic metabolism and drug hepatobiliary excretion in animal species and introduces some basic biochemical and pharmacokinetic concepts relevant to this role. Although these discussions are focused on the liver, the principles elucidated may also be applicable to extrahepatic sites of drug biotransformation.

Phase I and Phase II Reactions

Various metabolic pathways are involved in drug metabolism including oxidation, reduction, hydrolysis, hydration, and conjunction. These processes can be divided into Phase I and Phase II reactions (Table 2.2). Phase I includes reactions introducing functional groups to drug molecules necessary for the Phase II reactions, which primarily involve conjugation. In other words, Phase I products act as substrates for Phase II processes, resulting in conjugation with endogenous compounds, which further increase their water solubility and polarity, thus retarding tissue distribution and facilitating drug excretion from the body. Specific examples of drug metabolism are included in chapters throughout this text. The focus of this introduction will be to briefly overview the general processes involved in drug metabolism relative to how they might affect pharmacokinetic parameters and the disposition of drugs in the body. Interested readers should consult standard texts in drug metabolism or biochemical pharmacology/toxicology for specific detailed examples illustrating the chemistry and genetic control of these processes.

Our knowledge regarding the molecular mechanisms of drug metabolism has been predominately gained from studies on the liver at different experimental levels including in vivo intact animals; ex vivo liver perfusion; and in vitro liver slices, hepatocyte cell cultures, isolated/purified subcellular hepatocyte organelles, and isolated enzyme or enzyme components. Two subcellular organelles are quantitatively the most important; the endoplasmic reticulum (ER) (isolated in the microsome fraction) and the cytosol (isolated in the soluble cell fraction). Phase I oxidation enzymes are almost exclusively localized in the ER, along with the Phase II enzyme of glucuronyl transferase. In contrast, other Phase II enzymes are mainly present in the cytoplasm. Microsomal fractions of the hepatocyte retains most, if not all, of the enzymatic activity in drug metabolism.

Phase I metabolism includes four major pathways: oxidation, reduction, hydrolysis, and hydration, among which oxidation is the most important. Attention is usually focused on oxidation mediated by the microsomal mixed-function oxidase system (e.g., cytochrome P450, etc.) due to its central role and significance in governing the metabolic disposition of many drugs and xenobiotics. An understanding of this pathway is often critical to making interspecies extrapolations.

Phase II conjugating enzymes play a very important role in the deactivation of the Phase I metabolites of many drugs as well as in direct deactivation of some parent compounds when their specific structure doesn't require Phase I modification. For example, the analgesic drug paracetamol can be deactivated directly by Phase II reactions using glutathione, glucuronide, and sulfate conjugation mechanisms. Phase II deactivation can be achieved by both gross chemical modification of the drug thereby decreasing their receptor affinity, and by enhancement of excretion from the body, often via the kidney.

Among the reactions catalyzed by drug metabolism enzymes in the hepatic ER, cytochrome (Cyt) P450-dependent mixed-function oxidation is the most intensively studied. This reaction catalyzes the hydroxylation of hundreds of structurally diverse drugs and compounds, whose only common feature appears to be a relatively high lipophilicity. The enzyme consists of a family of closely related isoenzymes embedded in the ER membrane. Its name is based on the fact that the cytochrome is a pigment that exhibits a maximal absorbance wavelength of 450 nm when reduced and complexed with carbon monoxide. With the advent of gene cloning and sequencing, and the application of molecular biology techniques to Cyt P450 structure analysis, tremendous progress was made in the last decade in the isolation and sequencing of the cDNAs encoding multiple forms of the hemoprotein. The rapid determination of full-length Cyt P450 amino acid sequences enabled the development of a coherent nomenclature system describing hundreds of different and unique Cyt P450s.

A great deal of work has been fostered in this area through development of a nomenclature system for cytochrome P450 enzymes (CYP) based on DNA/amino acid sequence. This allows enzymes to be unambiguously classified. An enzyme is coded by its family (1,2,…) followed by subfamily (A–D) and then gene (1,2,…), and if necessary, allelic variant (*1, *2,…). Thus, a common

Table 2.3 Example of cytochrome P450 enzymes and substrates in the dog

Subfamily	Gene code	Sample substrates
1A	1A1, 1A2	Theophylline, phenacetin, 7-ethoxyresorufin, caffeine
2B	2B11	Ketamine, propofol, pentobarbital, warfarin
2C	2C21, 2C41	Diclofenic, midazolam
2D	2D15	β blockers, celecoxib, dextromethorphan
3A	3A12, 3A26	Macrolides, steroids, cyclosporine

enzyme found in dogs is termed CYP3A12, which is responsible for steroid oxidation. These enzymes are classified on the basis of sequence and not function. There is thus a great deal of overlap between substrate specificities and enzymes with different CYP identifications. The numbers are also species specific, thus preventing direct comparison between species. Not surprisingly, most work has been done for human enzymes, although recently work has also begun to define the primary enzymes involved in veterinary species. Table 2.3 lists some of the cytochrome P450 enzymes identified in the dog. This area of cytochrome P450 pharmacogenomics in the dog has recently been reviewed (Martinez et al., 2013).

Impact of Metabolism

One can precisely predict the impact of drug metabolism on therapeutic drug effect if one can identify the precise enzymes by which a drug is metabolized. The same enzymes are not involved in metabolizing the same drugs in different species and, in many cases, substrate specificities overlap, making precise prediction difficult. A variety of Phase I and Phase II reactions can take place simultaneously or sequentially in the body. For example, parathion can be catalyzed by Cyt P450 to an intermediate, which in turn can either be further oxidized to paraoxon or hydrolyzed to *p*-nitrophenol followed by conjugation reactions. Finally, as discussed earlier, a compound metabolized in the liver may be subsequently metabolized in the kidney prior to excretion, making it possible for these various metabolic steps to be carried out in multiple organs. Stereochemistry also plays a major role in drug metabolism since most enzyme system can be stereoselective. Examples include the enantiomers of amphetamine, cyclophosphamide, pentobarbitone, phenytoin, verapamil, and warfarin. Large differences in enzyme expression and function occur between species. Many of these were first identified in broad differences in the ability of some species studied to perform Phase II reactions (e.g., cats deficient in glucuronidation, pigs in sulfation). We now know that this is even more complex, especially in species and breed differences in specific CYP isoenzymes.

To further complicate this scenario, there is often overlap between cytochrome P450 substrate specificities and those for P-glycoprotein. That is, the same drug may be handled by both systems, classic examples being cyclosporine and ketoconazole. Such phenomena are often detected by complex and species-specific drug–drug interactions. For compounds handled in such a fashion, one can be assured that large differences will be seen between animal species making interspecies extrapolations very difficult.

In summary, Phase I metabolism is primarily responsible for drug deactivation, although Phase II plays an important role in deactivation of some drugs. Phase I reactions prepare drugs or toxicants for Phase II metabolism; that is Phase I modifies the drug molecule by introducing a chemically reactive group on which the Phase II reactions can be carried out for the final deactivation and excretion. This increased water solubility after metabolism restricts a drug's metabolite distribution to extracellular fluids, thereby enhancing excretion. Specific pathways for drug metabolism and transport are discussed in the individual drug chapters as well as their pharmacogenomics in Chapter 50.

Hepatic Clearance

As presented in our discussion on renal excretion, clearance of a drug by an organ ($\mathrm{Cl_{org}}$) can be ultimately defined as a function of its blood flow ($\mathrm{Q_{org}}$) and its extraction ratio ($\mathrm{E_{org}}$) expressed in Equation 2.6 as $\mathrm{Cl_{org} = Q_{org}\,E_{org}}$. The ability of the liver to remove drug from the blood, defined as *hepatic clearance*, is related to two variables: intrinsic hepatic clearance ($\mathrm{Cl_{int}}$) and rate of hepatic blood flow ($\mathrm{Q_h}$), as defined in Equation 2.13:

$$\mathrm{Cl_h = Q_h\left[Cl_{int}/\left(Q_h + Cl_{int}\right)\right] = Q_h E_h} \tag{2.13}$$

where Cl_h is the hepatic clearance, Q_h is the hepatic blood flow, and $Cl_{int}/(Q_h+Cl_{int})$ is the hepatic extraction ratio or E_h. Intrinsic clearance ($\mathrm{Cl_{int}}$) is conceptualized as the maximal ability of the liver to extract/metabolize drug when hepatic blood flow is not limiting. It represents the inherent metabolic function of all enzyme systems in the liver to metabolize the drug in question. As seen in Equation 2.13, when $\mathrm{Cl_{int} \gg Q_h}$, hepatic extraction ratio ≈1.0 (*flow limited or high extraction*, usually seen with $\mathrm{E_h > 0.8}$), $\mathrm{Cl_h}$ is dependent only on the blood perfusion rate $\mathrm{Q_h}$. The more blood passing through the liver, the more drug molecules will be extracted by the liver for metabolic elimination. A hepatic blood perfusion-dependent hepatic clearance will then be seen. Drugs with such high extraction ratios will show significant first-pass metabolism after oral administration to the

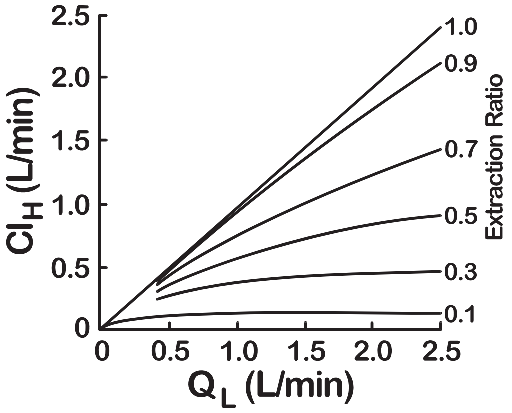


Figure 2.12 Relationship between liver blood flow (Q) and hepatic clearance (Cl_H) for drugs with different hepatic extraction ratios. Extraction ratio values are at a blood flow of 1.5 l/min.

extent that this route of administration may not produce effective systemic drug concentrations. Finally, the highest hepatic Cl possible is Q_h.

In contrast, if $Cl_{int} \ll Q_h$, the E_h is close to zero and thus the Cl_h is dependent only on the Cl_{int}; that is the liver extracts as many drug molecules as it can from the blood flow presented (*metabolism limited or low extraction*, usually with E_h <0.2). These two extremes occur with propranolol and antipyrine, respectively. Intermediate values of extraction ratio of 0.2–0.8 give hepatic clearance rates that can be dependent to varying extents on both hepatic blood perfusion rate and intrinsic clearance. Based on earlier discussions, one can appreciate that such classifications are very species specific since they depend upon the presence of specific enzyme systems for the specific drug.

To estimate hepatic drug clearance, one must consider the drug's physicochemical properties, hepatic drug metabolism enzyme activity, and rate of hepatic blood perfusion. This relationship between hepatic clearance and liver blood perfusion rate for drugs with different extraction ratios can be appreciated when Equation 2.13 is plotted in Figure 2.12. With a lower hepatic extraction drug, the blood perfusion rate is less important to $Cl_{hepatic}$. For a high hepatic extraction drug, the $Cl_{hepatic}$ is proportional to the blood flow, as discussed earlier. The reader should note the similarity of this discussion to that introduced earlier concerning capacity or flow-limited renal tubular clearance. The concepts are identical for both organs; however, they are more often employed when hepatic clearance is modeled due to the much greater difference in species-inherent metabolic capacities.

Metabolism Induction and Inhibition

Drug metabolism is substantially influenced by enzyme induction or inhibition that occurs secondary to the deliberate or passive intake of a number of chemicals that animals are increasingly exposed to either in the environment, for medical reasons, as dietary supplements, or in humans simply as a result of lifestyle (smoking, alcohol consumption, etc.). In laboratory animals, contaminants and natural constituents of diet have been shown to affect the pattern of drug metabolism observed. In many cases, the compound itself may alter its own metabolic fate by induction or inhibition.

Induction

Many currently used drugs, food additives, household chemicals, and environmental contaminants (including pesticides) possessing diverse chemical structure, pharmacological or toxicological activity are well known to induce their own metabolism and/or that of other compounds in humans and animals. Induction of metabolism may arise as a consequence of increased synthesis (at different transcriptional/translational levels), decreased degradation, activation of preexisting components, or a combination of these processes. With so many compounds able to alter hepatic metabolism, a great deal of effort has been spent in recent years to understand the mechanisms behind these processes. This is important from a therapeutic perspective since the intrinsic hepatic clearance of a drug will change if the enzymes responsible for metabolizing it are induced, thereby increasing metabolic capacity. Similarly, the pattern of Phase I and Phase II metabolism may be changed if one enzyme component's activity has been modified by inducers. These interactions introduce a significant complexity to pharmacokinetic models describing the disposition of drugs extensively metabolized by the liver. However, they have also prompted research efforts aimed at elucidating the mechanisms behind these processes, which, when understood, should provide a strategy for developing mechanistically meaningful models for simulation of drug metabolic disposition.

As discussed above, the activity of the Cyt P450 system is of particular importance to the overall hepatic metabolic clearance of a drug. In the mid-1960s, both Cyt P450 and its associated flavoprotein reductase were found to be induced by phenobarbitone pretreatment that was accompanied by induction of drug metabolism. Induction was generally accompanied by increases in liver microsomal Cyt P450 content. Diverse drug metabolism responses to different inducers, which all induce hepatic Cyt P450, can be dependent on the substrate of interest (substrate specificity) with stero- and regioselectivity, confirming that subpopulations of Cyt P450 (isoenzymes) might be present. This now widely accepted concept has had a profound influence on drug discovery, design of metabolism studies, and the resulting structure of pharmacokinetic models. With the advent of the CYP nomenclature system discussed above, enzymes can now be classified both as to substrate specificity, but also as to which compounds induce or inhibit their function. Pharmacogenomic studies have begun to identify the specific genes responsible for isoenzyme induction and also identify species differences. Unfortunately, these studies are just beginning to be applied in veterinary species and a complete picture is still lacking on their impact on clinical therapeutics. A major cause of this problem is that, as mentioned above, there are not specific homologs between human and veterinary species enzymes, making marker substrates different. The important concept is that induction will occur and could have a significant impact on therapeutic efficacy. Specific examples of such interactions are presented within the drug-specific chapters of this text.

Metabolism Inhibition

Similar to the induction of metabolism, inhibition is a well-recognized phenomenon secondary to serial drug dosing, coadministration of drugs, endogenous compounds, environmental xenobiotics, and complex multiple-ingredient drug formulations. Several mechanisms for metabolism inhibition have been noted, including the destruction of preexisting enzymes (by porphyrinogic drugs and xenobiotics containing olefinic ($C = C$) and acetylenic ($C \equiv C$) functions), inhibition of enzyme synthesis (by metal ions), or complexing with the hemoprotein thereby inactivating enzymes. Many drug–drug interactions may be explained at the level of Cyt P450 destruction. In contrast to the porphyrinogic drugs, metal ions such as cobalt exert their inhibitory effects by modulating both the synthesis and degradation of the heme prosthetic group of Cyt P450. Formation of inactive Cyt P450-inhibitor complex is another mechanism for drug metabolism inhibition. Inhibitors are usually substrates of Cyt P450 and require metabolic conversion to exert their full inhibitory effects, in a manner similar to porphyrinogic drugs and xenobiotics. However, inhibitors forming complexes with hemoprotein are metabolized by Cyt P450. These inhibitors can form metabolic intermediates or products that tightly bind to the hemoprotein, thereby preventing its further participation in drug metabolism. As can occur with induction, coadministration of inhibitor drugs may result in clinically important drug interactions. Specific examples of such interactions are presented within the drug-specific chapters of this text.

Biliary Drug Elimination

As an exocrine function of the liver, bile excretion is thought to be present in almost all vertebrates. The three basic physiological functions of the bile are (i) to serve as the excretory route for products of biotransformation, (ii) to facilitate the intestinal absorption of ingested lipids such as fatty acids, cholesterol, lecithin, and/or monoglycerides due to the surfactant properties of bile forming mixed micelles, and (iii) to serve as a major route for cholesterol elimination in order to maintain normal plasma cholesterol levels. In addition to its physiological functions, bile is also pharmacologically and toxicologically important since some heavy metals and enzymes are also excreted via the biliary system. Bile secretion is very important to chemical/drug transport and elimination under both physiological and pathological conditions. However, bile secretion has proven difficult to study mainly due to the inaccessibility of the biliary tree for direct sampling.

The Mechanism of Bile Formation

Bile is continuously produced by liver cells and then stored in the gall bladder, except for those species (rat, horse) lacking it. The pH of bile ranges from 5.0 to 7.5 depending mainly on the animal species. Biliary excretion is a major route for some drugs with MW >300 and a high degree of polarity. This occurs by active transport of drug and metabolites into bile, thus saturation and competition are important issues to consider. Passive diffusion of drug into bile is insignificant. Most of the compounds secreted into bile are finally excreted from the body in feces where they may be subject to enterohepatic circulation and degradation by intestinal microflora.

Bile is formed at two sites: the ramifications of the bile duct within the portal triads and the anastomosing network of the narrow bile canaliculi in the hepatic parenchyma. The bile canaliculi are the primary secretory units of the liver. These small channels or furrows are lined by the apical membranes of the hepatocytes and thus do not have their own epithelium or basement membrane. Because hepatocytes form a canalicular lumen wherever they abut, most canaliculi communicate with each other, forming an anastomosing network. Similar to the relationship of the nephron to the kidney, the volume and composition of canalicular bile are often determined by the activity of several cords of hepatocytes.

The overall bile flow is in the opposite direction to sinusoidal blood flow and thus solute transfer from plasma to bile involves a counterflow process (Figure 2.13). Such a blood–bile flow pattern reduces rediffusion of biliary solutes such as drugs and metabolites back into sinusoidal plasma in the portal area, which is richer in solute concentration, bathes periportal hepatocytes, and is exposed to higher canalicular concentration of any given solute. Three routes of fluid and drug transfer from the sinusoid to the bile canaliculus have been postulated: transcellular, paracellular, and vesicular. These multiple mechanisms contribute to the large interspecies differences seen in biliary drug excretion.

Drug uptake into hepatocytes by passive diffusion is so efficient that it is rate limited by the delivery of the

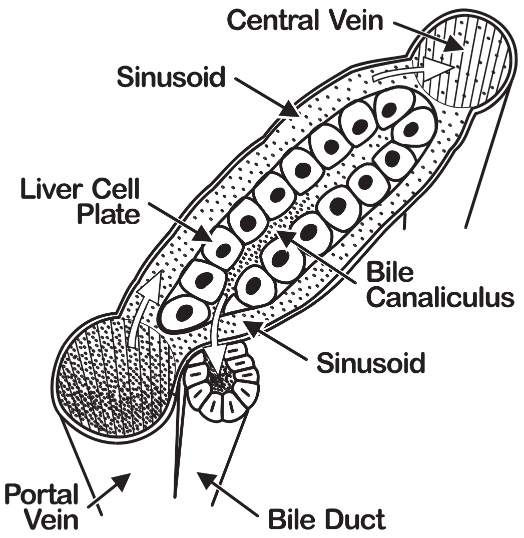


Figure 2.13 Gradient concept of bile secretion in a liver lobule.

drug to the liver (i.e., blood flow) rather than membrane transport, thereby exhibiting flow-dependent clearance. However, for highly polar molecules, passive diffusion is not an efficient mode of hepatocellular uptake, and there is an increased reliance on carrier-mediated transport systems. Drug metabolites, particularly conjugated metabolites (e.g., sulphates and glucuronides), are invariably more polar than their precursors and thus are more likely to experience hepatocyte membranes as diffusional barriers. With such a barrier, the hepatocellular export of a locally formed metabolite will depend on the presence and activity of carrier-mediated transport systems for sinusoidal efflux and biliary excretion. Transport systems of current interest include the P-glycoproteins, which are responsible for the biliary excretion of a range of organic cations, and the canalicular multispecific organic anion transporter. Intracellular trapping of metabolites formed in the liver, secondary to low membrane permeability, is clinically important because many are potentially hepatotoxic and/or capable of interfering with the hepatic transport of endogenous compounds or other drugs and metabolites. Again this phenomenon is conceptually similar to renal tubular sequestration and has similar pharmacokinetic implications. Finally, if the metabolite is unstable, intracellular accumulation can lead to the regeneration of the precursor and so-called “futile cycling” within hepatocytes.

Biliary Drug Transport

Some parent drugs and numerous drug metabolites derived from hepatic metabolism are excreted in the bile into the intestinal tract. The excreted metabolites can be excreted via feces although, more commonly, they are subject to reabsorption into the blood and are eventually excreted from the body via urine. There are at least three different biliary transport pathways for organic anions, cations, and neutral compounds, although metals may also have their own transport carriers/systems. Both *organic anions* and *organic cations* can be actively transported into bile by carrier systems again similar to those involved in the renal tubule. Such transport systems are nonselective, and ions with similar electrical charge may compete for the same transport mechanisms. Additionally, a third carrier system, whose activity is sex dependent, may be involved in the active transport of *steroids* and *related compounds* into bile. In contrast to renal excretion, amphiphatic drugs (those having both polar and nonpolar properties) are preferentially excreted in the bile. The drug (or metabolite) excreted into the small intestine can be reabsorbed into blood forming the so-called drug enterohepatic cycle. This is an important factor changing the blood : liver or liver : bile drug concentration ratios during studies of the hepatobiliary transport mechanisms and drug hepatic elimination.

The biliary excretion of weak acids is the most important mechanism in drug hepatic elimination. Bromosulfophtalein (BSP) and analogues are dyes used as diagnostic probes of liver function and model substances in studies of the hepatic uptake of organic anions. Antibiotics such as ciprofloxacin can be actively excreted into bile in the presence of a biliary tract obstruction. Tetracyclines, mainly excreted into urine via glomerular filtration, are also concentrated in the liver and accordingly excreted into the small intestine via the bile, and then partially reabsorbed.

Glucuronides of endogenous compounds and drugs can be actively transported from hepatocytes into bile via transport systems similar to those for organic anions. Glucuronide conjugates are very important in hepatic drug metabolism and biliary excretion. The effectiveness of biliary excretion for glucuronide conjugates can be greatly limited by enzymatic hydrolysis after the bile is mixed with the small intestine contents, thereby releasing the parent drugs to be reabsorbed and enter the enterohepatic cycle. The reabsorbed drugs and metabolites can be ultimately excreted in urine. Some drug metabolites further undergo either biotransformation in the liver or other organs or are subjected to microbiological and physicochemical degradation in the small intestine before being excreted in the feces.

Weak bases can be actively transported into bile via carrier systems similar to the renal transport processes. Atropine, isoproterenol, and curare are eliminated by this mechanism, with atropine being almost equally excreted by the kidney (unchanged form) and hepatic metabolism followed by biliary excretion. Organic cation transport is not as important as the organic anion pathway. Neutral compounds may employ the third transport systems. Ouabain, a cardiac glycoside, is used as a model uncharged and nonmetabolized (by rat liver) compound

Table 2.4 Mean bile flow in selected species

Species	Bile flow (ml/min/kg body weight)
Cat	11
Chicken	20
Dog	4–10
Guinea pig	200
Hamster	50
Human	5–7
Monkey	10
Mouse	78
Opossum	20
Pig	9
Pony	19
Rabbit	90
Rat	50–80
Sheep	43

in hepatobiliary transport studies. Organic anions and neutral steroids such as ouabain may share common mechanisms in their excretory pathways.

Molecular weight is a key determinant of the extent to which drug/metabolite molecules are transported into bile. The molecular weight cutoff required for biliary excretion is much greater than that for renal tubular secretion, being from 300 to 500 in most species. If the molecular weight is lower, the compound may be preferentially excreted in urine. Excretion of molecules larger than 850 Da occurs mainly via the biliary active transport system. However, molecular weight is not the sole factor determining the route of drug excretion. Physicochemical properties of the drug (polarity/lipophilicity, structure) are also very critical to the extent of biliary excretion of a drug/metabolite, with amphiphatic drugs being well secreted by the biliary route.

The specific animal species being studied is also an important factor, as reflected in different molecular weight cutoff thresholds. Table 2.4 lists species differences in bile flow rate that may also contribute to the great species specificity seen in biliary drug transport. Such variations in both a species' enzyme profile for biotransformation and inherent ability to excrete drugs in bile result in great difficulty in predicting hepatic clearance across drugs in different species.

Conclusion

This chapter presents an overview of some essential principles of ADME that are needed to understand drug- and species-specific behavior of drugs, examples of which are discussed in most chapters of this text. The next chapter focuses on the pharmacokinetic tools used to quantitate these processes so that safe and effective dosages of drugs can be administered to animals. There is a tremendous amount of research being conducted on the determinants of ADME processes because they are so important in assuring safety and efficacy of drugs across all species. They are the primary source of differences in drug action across species.

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3

Pharmacokinetics

Jim E. Riviere

Pharmacokinetics is best defined as *the use of mathematical models to quantitate the time course of drug absorption and disposition in man and animals*. With the tremendous advances in medicine and analytical chemistry, coupled with the almost universal availability of computers, what was once an arcane science has now entered the mainstream of most fields of human and veterinary medicine. This discipline has allowed dosages of drugs to be tailored to individuals or groups to optimize therapeutic effectiveness, minimize toxicity, and avoid violative tissue residues in the case of food-producing animals. This subject and its concepts have become especially important as a consequence of the dramatic and almost radical changes that occurred at the end of the last decade relative to the regulations surrounding drug use in veterinary medicine in the United States. For most of the recent past, the operative concept was that a single dose of drug listed on a product label was optimal for all therapeutic uses. Recently, however, the legal concept of "flexible or professional labeling" and the passage by the US Congress in 1994 of the Animal Medicinal Drug Use Clarification Act (AMDUCA) legalizing extralabel drug use challenged this simplistic ideal of a single optimal dose. The veterinarian must now select a drug dose based on numerous factors inherent to the therapeutic scenario at hand to maximize therapeutic efficacy and minimize the likelihood of drug-induced toxicity or induction of microbial resistance. Furthermore, linking pharmacokinetic models to models of drug action, presented in the next chapter, allows for developing a quantitative link between dose and efficacy or toxicity. Unlike human medicine and companion animal practices, food animal veterinarians face the further restriction that proper withdrawal times must be determined to ensure that drug residues do not persist in the edible tissues or by-products (milk, eggs) of treated animals long after they have left the care of the veterinarian (Figure 3.1). As will be demonstrated and further discussed in Chapter 61, the "withdrawal time" is in reality a pure pharmacokinetic parameter since it can be calculated solely from a knowledge of the legal tissue tolerance and the drug's half-life or rate of decay in that tissue.

Yet it is not only the food animal veterinarian that faces these challenges. The laboratory animal and exotic/zoo animal worker must often extrapolate drug dosages across species with widely differing body sizes and physiology since there are very few approved drugs for the treatment of such animals. Pharmacokinetic principles and techniques are ideally suited for this application. Practitioners are often faced with disease processes (e.g., renal failure) that are known to affect the disposition of a drug. Knowledge of how such a pathological process affects a drug's clearance is sufficient knowledge to adapt a dosage regimen appropriate for this condition.

The previous chapter of this text presented the underlying physiology of drug fate. The processes involved in absorption, distribution, metabolism, and elimination are the primary phenomena that must be quantitated to predict the fate of a drug or toxicant in an animal. The two primary characteristics needed to adequately describe these processes are their *rate* and *extent*. In fact, this can be appreciated in the origin of the word *kinetic,* which is defined as: "*of or resulting from motion*." Many mathematical approaches to this problem have evolved over the course of the history of pharmacokinetics. The reader is suggested to consult the selected readings for more in depth presentations of pharmacokinetic modeling. In addition, hybrid as well as novel strategies are constantly being developed to quantitate these processes. However, all approaches share certain fundamental properties that are based upon estimating the rates of chemical movement.

A Primer on the Language of Pharmacokinetics

The roots of pharmacokinetics lie in the estimation of rates. The language is that of differential calculus. It is instructive to briefly overview the basic principles of rate

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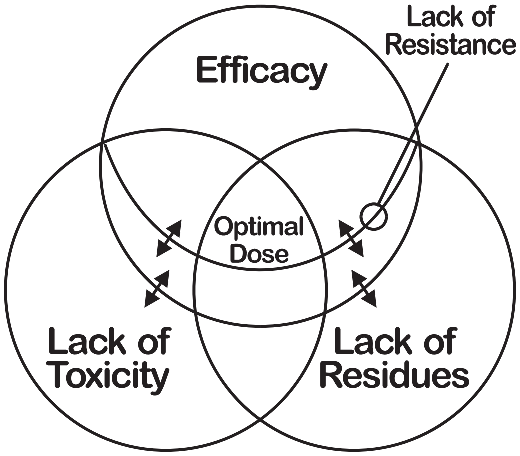

Figure 3.1 The food animal veterinarian's dilemma in optimizing the dose of a therapeutic drug.

determination since the logic imbedded in its syntax forms the basis of pharmacokinetic terminology.

To begin, a *rate* in pharmacokinetics is defined as *how fast the mass of a compound changes per unit of time*, which is expressed mathematically as the change (represented by the Greek letter delta, Δ) in mass per small unit of time (Δt). This is synonymous with the flux of drug in a system. Units of rate are thus mass/time. For the sake of convenience only, we will express this in terms of mg/min. We will begin this discussion using mass of a compound (X), which in clinical terms would be related to the dose, rather than using concentration. As will be developed shortly, mass and concentration are easily convertible using the proportionality factor of volume of distribution.

The rate of drug excretion ΔX/Δt actually has two components, a constant that reflects the rate of the process and the amount of compound available for transfer (Equation 3.1):

$$\Delta X/\Delta t = KX^n \tag{3.1}$$

where K is the fractional rate constant (1/min), X (mg) is the mass or amount of a compound available for transfer by the process being studied, and n is the order of the process.

For a first-order process, n = 1. Since $X^1 = X$, this equation simplifies to Equation 3.2:

$$\Delta X/\Delta t = KX \tag{3.2}$$

By definition, in this first-order (n = 1) or linear processes, K is *constant* and thus *the actual rate of the process ($\Delta X/\Delta t$) varies in direct proportion (and hence linearly) to X*. K can be viewed as the fraction of X that moves in the system being studied (absorbed, distributed, or eliminated) per unit of time. Therefore, as X increases, ΔX/Δt increases in direct proportion. In linear models, the rate constant is fixed but the rate of the process changes in direct proportion to the mass available for movement.

As can again be appreciated by examining the equation for Fickian diffusion (Equation 2.1 in Chapter 2), compounds that are either absorbed, distributed, or eliminated in direct proportion to a concentration gradient are by definition first-order rate processes. The rate constants (K_n) modeled in pharmacokinetics are actually aggregate constants reflecting all of the membrane diffusion and transfer processes involved in the disposition parameter being studied. This includes pH partitioning phenomena in the body, which exist when blood and a cellular or tissue compartment have a pH gradient that alters the fraction of drug available for diffusion. Recall that it is only the unionized fraction of a weak acid or base that diffuses down its gradient across a lipid membrane. The rate constant also reflects the degree of plasma protein binding since only the free fraction of drug is available for distribution. The actual value of a K in a pharmacokinetic model thus reflects all of these variables whose relationship defines the biological system that we are attempting to quantitate.

For a nonlinear or zero-order process, by definition n = 0. Since $X^0 = 1$, the rate equation now becomes

$$\Delta X/\Delta t = K_0 \tag{3.3}$$

In this scenario, the *rate of excretion is fixed and thus independent of the amount of compound available, X*. K_o now has the units of rate (mg/min) and is *not* a mass-independent fractional rate constant. Although this would appear to simplify the situation, in reality nonlinear kinetics actually complicate most models. Nonlinear behavior becomes evident when saturation of a process occurs. The focus of most pharmacokinetic studies is on drugs with linear pharmacokinetics since the majority of therapeutically active compounds are described by these models.

The use of ΔX/Δt to describe the rate of a process is experimentally and mathematically cumbersome, since ΔX/Δt changes as a function of concentration. Figure 3.2 graphically depicts this scenario. Calculus has been used to describe these same processes using the concept of a derivative. Instead of describing rates in terms of some small, finite time interval (Δt), differential equations express rate in terms of the change in compound mass over an infinitesimally small time interval termed dt. Equation 3.1 could now be written as the following (Equation 3.4):

$$dX/dt = -KX \tag{3.4}$$

The biological interpretation is identical. Note that K and X are the same in both equations; the only change is a conceptual one in that *dX/dt* now describes the instantaneous rate of change in mass over time. By convention, if the amount of drug is increasing, dX/dt is positive (e.g.,

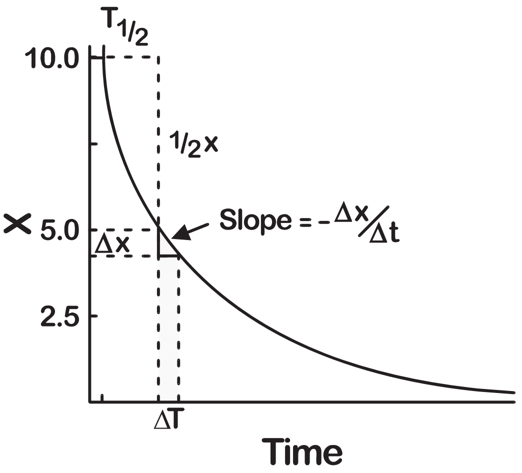

Figure 3.2 Plot of the decay in drug (X) versus time. The T1/2 is defined as the time required for X to deplete to 1/2 X. Slope at any time is ΔX/ΔT.

absorption of drug into the blood); if it is declining (e.g., elimination or distribution from the blood), the rate is negative or −dX/dt.

One may solve a differential equation through the process of integration ($\int$), which transforms the equation back into terms of t rather than dt. Integration is in reality a process by which the area under the curve (AUC) defined by ΔX/Δt is taken. By repeatedly summing these areas for the entire experimental period, the area under the curve will be obtained. We introduced this concept in Equation 2.4 when the concept of AUC was introduced. Analogous to the relation of a derivative to a slope, integration sums the areas under infinitesimally small regions defined by dX/dt.

We can use the technique of integration to solve the rate Equation 3.4. We must integrate the equation from X at time zero (X_0) through X at time t (X_t) to obtain a formula for the mass of drug at any time (Equation 3.5):

$$\int_0^t \left(\frac{dX}{dt}\right) dt = \int_0^t (-KX)\ dt \tag{3.5}$$

There are numerous techniques to accomplish this integration and the interested reader should consult a calculus textbook for further details. The result is the following (Equation 3.6):

$$X_t = X_0 e^{-Kt} \tag{3.6}$$

where *e* is the base of the natural logarithm (e = 2.713). *It is important to realize that the process of integrating the differential equation describing rate generates the exponential term found in most linear pharmacokinetic models.* Exponentials can easily be eliminated from an equation by taking their natural logarithm (*ln*) since the logarithm is defined as the power to which a base (in this case *e*) is raised. Taking the natural logarithms of Equation 3.6 yields the following (Equation 3.7):

$$\ln X_t = \ln X_0 - Kt \tag{3.7}$$

If one plots these data, a straight line results, as seen in Figure 3.3, which is much easier to deal with than the curve in Figure 3.2. Recalling the algebraic expression for a straight line on x–y coordinates, in this case the y intercept becomes X_0 and the slope of the line is −K. The equation has been linearized providing a simple graphical method to calculate the rate constant.

This equation can be linearized because it is a first-order rate function. This type of plot, which is widely used throughout pharmacokinetics, is termed a semilogarithmic plot (in contrast to the Cartesian plot) since the logarithm of mass is plotted against time. Again, *when a straight line results on a semilogarithmic plot, one can assume that a linear first-order process is operative and the slope of the line is the exponent of an exponential equation.* Alternatively, a linear regression program on a computer or calculator could be used to calculate K by regressing the ln X against time; the slope is −K and the y intercept is $\ln X_0$. In earlier work, graph paper was used that plotted logarithms to the base 10 (log x) $\{10^x\}$ rather than the base e (ln x) $\{e^x\}$ where x is the logarithm, the transformation of bases can be accomplished as the following (Equation 3.8):

$$\log X = \ln X/2.303 \tag{3.8}$$

which transforms Equation 3.7 to

$$\log X_t = \log X_0 - Kt/2.303 \tag{3.9}$$

If base 10 semilogarithmic graph paper is used to plot Figure 3.3, the slope becomes −K/2.303. As this technique was in widespread use before the advent of digital computers, this is still encountered in older manuscripts and texts.

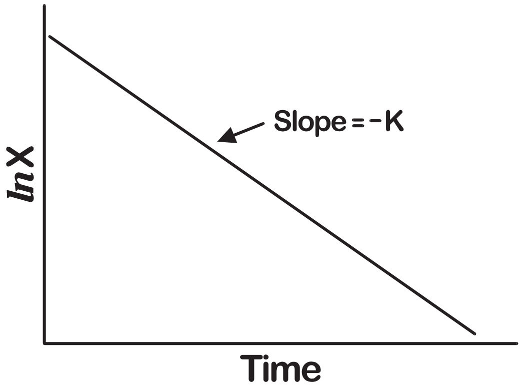

Figure 3.3 Semilogarithmic plot of drug decay versus time with slope equal to −K.

The Concept of Half-Life

The exponential equations in pharmacokinetics have another property that is important to biological applications. This is the concept of half-life (T1/2) whose logic is central to much of this discipline. The astute biologist reading this text will have realized that Equation 3.6 is the same as that used to describe population doubling times in microbiology or ecology and used to generate population growth curves, defined as the time needed for a population of organisms to double their total numbers when they are in their so-called *logarithmic* growth phase. The only difference is that since growth is described, the exponent is positive in this application. In pharmacokinetics, our perspective is decay with a T1/2 instead being the time required for the amount of drug to decrease by one-half or 50%. The concept of T1/2 is applicable only to first-order rate processes.

Using Equation 3.7, one can derive a simple equation for T1/2. We first rearrange terms to solve for T, which yields

$$T = (\ln X_0 - \ln X_t)/K \tag{3.10}$$

We now solve for the time at which X_t is equal to 1/2 the initial amount X_0, that is, where $T =$ T1/2. Substituting these values, the equation reduces to

$$K = 0.693/T{}^{1}\!/_{2}. \tag{3.11}$$

It is this transformation of K with T1/2 that introduces the ln 2 or 0.693 into many pharmacokinetic equations.

What does T1/2 really mean? Assume that we start with X, decrease it by half, and repeat this process 10 times. Table 3.1 compiles this data and lists how much drug is remaining and how much has been excreted over each Δt corresponding to one T1/2. Note that if you sum these columns, you would have accounted for 99.9% of the original dose X. After 10 T1/2s, 99.9% of the drug has been eliminated or the rate process being studied has been completed. This also illustrates the logic that must be used when dealing with doses. For example, if you double the dose to 2×, then after one T1/2 you would be back to the original dose! Many rules of thumb used in pharmacokinetics and medicine are based on this simple fact. For therapeutic drugs, most workers assume that after five T1/2s, the drug has been depleted or the process is over since 97% of the depletion has occurred. This also illustrates a very simple way to calculate T1/2, by simply determining the time required for drug concentration to decrease by 50%. This was depicted in Figure 3.2. Equation 3.11 can then be used to obtain K. It now is time to develop our first pharmacokinetic model using mathematical rather than physiological concepts.

Table 3.1 Relationship of T1/2 and amount of drug (A) in the body

Number of T1/2s	% of Drug remaining	% of Drug eliminated
1	50	50
2	75	25
3	87.5	12.5
4	93.75	0.625
5	96.88	0.312
6	98.44	0.156
7	99.22	0.078
8	99.61	0.039
9	99.80	0.019
10	99.90	0.0097

One-Compartment Open Model

The most widely used modeling paradigm in comparative and veterinary medicine is the compartmental approach. In this analysis, the body is viewed as being composed of a number of so-called equilibrium compartments, each defined as representing nonspecific body regions *where the rates of compound disappearance* are of a similar order of magnitude. Specifically, the fraction or percent of drug eliminated per unit of time from such a defined compartment is constant. Such compartments are classified and grouped on the basis *of similar rates of drug movement* within a kinetically homogeneous but anatomically and physiologically heterogeneous group of tissues. These compartments are theoretical entities that allow formulation of mathematical models to describe a drug's behavior over time with respect to movement within and between compartments. Since pharmacologists and clinicians sample blood as a common and accessible biological matrix for assessing drug fate, most pharmacokinetic models are constructed with blood or plasma drug concentrations as the central reference to which other processes are related.

The simplest compartment model is when one considers the body as consisting of a single homogeneous compartment; that is, the entire dose X of drug is assumed to move out of the body at a single rate. This model, depicted in Figure 3.4, is best conceptualized as instantly dissolving and homogeneously mixing the drug in a beaker from which it is eliminated by a single rate process described by the rate constant K, now termed K_{el}. Since the drug leaves the system, the model is termed *open*. Equation 3.6 is the pharmacokinetic equation for the one-compartment open model. Although expressed in terms of the amount of drug remaining in the compartment, most experiments measure concentrations. This requires the development of the volume of distribution (Vd) (recall Equation 2.5 when distribution was discussed). In terms of the one-compartment model, this would be the volume of the compartment into which the dose of drug (D) instantaneously distributes. *Vd*

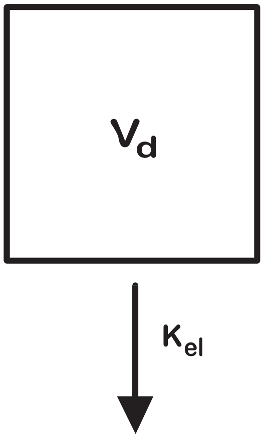

Figure 3.4 One-compartment open pharmacokinetic model.

thus becomes a *proportionality factor* relating *D* to the observed concentration *Cp* by

$$Vd\ (ml) = X\ (\cancel{mg})/Cp\ (\cancel{mg}/ml) = D/Cp \qquad (3.12)$$

Using this relation, we can now rewrite Equation 3.6 in terms of concentrations, which are experimentally accessible by sampling blood, instead of the total amount of drug remaining in the body.

$$Cp = X_0/Vd\ e^{-Kelt} = Cp_0 e^{-Kelt} \qquad (3.13)$$

A semilogarithmic plot seen after intravenous administration using this model is depicted in Figure 3.5. Vd quantitates the apparent volume into which a drug is dissolved, since, recalling the discussion in Chapter 2, the true volume is determined by the physiology of the animal, the relative transmembrane diffusion coefficients, and the chemical properties of the drug being studied. A drug that is restricted to the vascular system will have a very small Vd; one which distributes to total body water will have a very large Vd. In fact, it is this technique that is used to calculate the plasma and interstitial spaces.

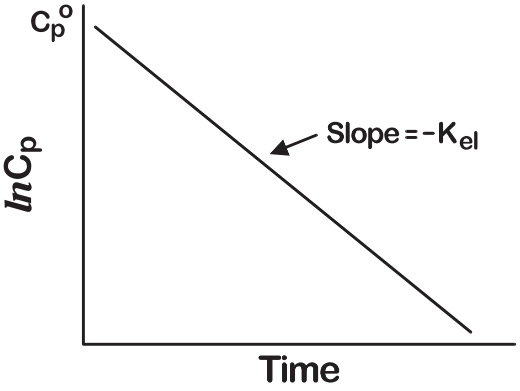

Figure 3.5 Semilogarithmic concentration-time profile for a one-compartment drug with slope $-K_{el}$ and intercept Cp_0.

From this simple analysis, and using the model in Figure 3.4, a number of useful pharmacokinetic parameters may be defined. Assuming that an experiment such as depicted in Figure 3.5 has been conducted using a dose of D and values for K_{el} and Vd have been determined, T1/2 can easily be calculated from Equation 3.11 above.

Clearance

Recalling the development of clearance concepts in Chapter 2, we now can easily determine Cl_B using this information. Clearance was defined as *the volume of blood cleared of a substance by the kidney per unit of time.* If one considers the whole body, this would read as *the volume of distribution of drug in the body cleared of a substance per unit of time.* Translating this sentence to the syntax of pharmacokinetic terminology and considering whole body elimination, Vd represents the volume and K_{el} the fractional rate constant (units of 1/time). Thus clearance is

$$Cl_B(ml/min) = Vd)ml)\ K_{el}(1/min) \qquad (3.14)$$

There is another method available to calculate Cl_B. In Chapter 2, clearance was also defined in Equation 2.8 as *the rate of drug excretion relative to its plasma concentration*. We can also express this sentence in the syntax of pharmacokinetics and get this relation:

$$Cl_B = (dX/dt)/Cp \qquad (3.15)$$

If we integrate both the numerator and denominator of this relation from time 0 → ∞, the numerator is the sum of the total amount of drug which has been excreted from the body; that is, assuming intravenous administration, the administered dose D. The denominator is the integral of the plasma concentration time profile the area under the curve (AUC). The relation thus becomes:

$$Cl_B = D/AUC \qquad (3.16)$$

There are two approaches to calculate AUC. A common approach is to use the trapezoidal method depicted in Figure 3.6. However, for the one-compartment model that generates the semilogarithmic C-T plot depicted in Figure 3.5, the problem is simply determining the area of the right triangle. The area of this triangle (AUC) is height divided by the slope of the hypotenuse, or:

$$AUC = Cp_0/K_{el} \qquad (3.17)$$

Interpretation of Pharmacokinetic Parameters

With these equations, we now have the three so-called *primary pharmacokinetic* parameters describing drug disposition in the body: *T*1/2, Cl_B, and *Vd.* The data required to calculate them is a knowledge of dose and an experimental derivation of either K_{el} or T1/2.

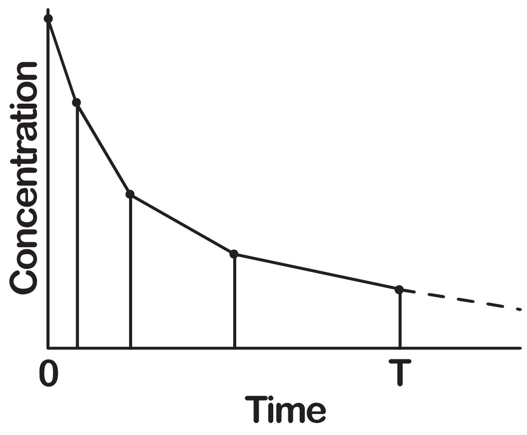

Figure 3.6 Trapezoids formed from sampled concentration versus time data for calculation of areas. To estimate the total AUC, the curve must be extrapolated beyond the ultimate sample time, T, to infinity (—).

This is a good place to discuss the limits of calculating parameters from such simple concentration-time profiles. Only two parameters are actually being "measured" from this analysis: the slope K_{el} and intercept Cp_0 of the semilogarithmic plot, which – using Equation 3.12 directly – determines Vd. The third parameter Cl is "calculated" from the two measured parameters. Based on the mathematical method used to calculate these, some workers suggested that K_{el} and Vd are the independent parameters in a pharmacokinetic analysis and Cl is a derived parameter. This assertion is usually made when the statistical properties of the parameters are being defined since errors for these can be easily obtained. However, this belief is an artifact of the use of a compartmental model as a *tool* to get at values for these physiological parameters. Biologically, the truly independent parameters are the Vd and Cl, with K_{el} and thus T1/2 becoming the dependent variables. From this biological perspective, the true relationship is

$$T1/2 = (0.693 \times Vd)/Cl \tag{3.18}$$

The observed half-life of a drug is dependent upon *both* the extent of a drug's distribution in the body *and* its rate of clearance. If the clearance of a drug is high (e.g., rapidly eliminated by the kidney), the T1/2 is relatively short. Logically, a slowly eliminated drug will have a prolonged T1/2. Not obvious at first is that if a drug is extensively distributed in the body (e.g., lipid-soluble drug distributed to fat), Vd will be large *and* the T1/2 will also be relatively prolonged. In contrast, if a drug has restricted distribution in the body (e.g., only the vascular system), the Vd will be small, a large fraction of the drug will be available for elimination, and thus the T1/2 relatively short. In a disease state, T1/2 may be prolonged by either a diseased kidney, a reduced capacity for hepatic drug metabolism, or an inflammatory state, which increases capillary perfusion and permeability, thus allowing drug access to normally excluded tissue sites. Therefore, T1/2 is physiologically dependent on both the volume of distribution and clearance of the drug.

Cl_B is the sum of clearances from all routes of administration:

$$Cl_B = Cl_{Renal} + Cl_{Hepatic} + Cl_{other} \tag{3.19}$$

There is another strategy that can be used to estimate clearance in an intravenous study. This is based on the basic principle of mass balance. The strategy is to infuse a drug into the body at a constant rate R_o (mass/time) and then measure plasma drug concentrations. By definition, when a steady-state plasma concentration is achieved, C^{ss} (mass/volume), the rate of drug input must equal the rate of clearance from the body, Cl_B:

$$R_o\ (mg/\text{min}) = C^{SS}\ (mg/\cancel{ml}) \times Cl\ (\cancel{ml}/\text{min}) \tag{3.20}$$

Rearranging this equation gives a simple formula for determining Cl:

$$\begin{aligned} Cl\ (ml/\text{min}) &= R_o\ (\cancel{mg}/\text{min})/C^{SS}\ (\cancel{mg}/ml) \\ &= R_o/C^{SS}\ (ml/\text{min}) \end{aligned} \tag{3.21}$$

The Cl calculated in this manner is identical to that determined using Equations 3.14 and 3.16 above, and requires only knowing the rate of infusion and assaying the achieved steady-state concentration. One may also calculate the Vd from an intravenous infusion study by the relation:

$$Vd = R_0/C^{SS}K_{el} \tag{3.22}$$

Many of the pharmacokinetic parameters above may also be obtained by analysis of urine data alone, an approach beyond the focus of the present introduction.

Absorption in a One-Compartment Open Model

The analysis above assumes that the drug was injected into the body, which behaves as a single space into which the drug is uniformly dissolved. The first real-world complication is when the drug is administered by one of the extravascular routes discussed in Chapter 2. In this case, the drug must be absorbed from the dosing site into the bloodstream. The resulting semilogarithmic concentration–time profile, depicted in Figure 3.7, now is characterized by an initial rising component that peaks and then undergoes the same log-linear decline. The proper pharmacokinetic model for this scenario is depicted in Figure 3.8. The rate of the drug's absorption is governed by the rate constant K_a. When the absorption process is finally complete, elimination is still described by K_{el} as depicted in Figure 3.5. The overall elimination half-life can still be calculated using K_{el} if this terminal slope is taken after the peak (C^{max}) in the linear portion of

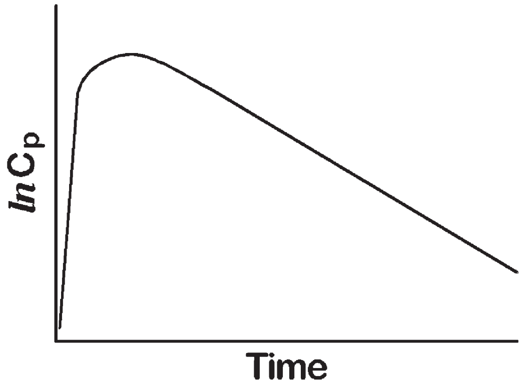

Figure 3.7 Semilogarithmic plot of plasma concentration versus time with first-order absorption.

the semilogarithmic plot (providing $K_a \gg K_{el}$). However, calculation of Vd and Cl becomes more complicated since K_a is present and, unlike an intravenous injection, one is not assured that all of the drug has been absorbed into the body. In order to handle this, we must now write the differential equations to describe this process by including rate constants for absorption and elimination:

$$dX/dt = K_aD - K_{el}X \tag{3.23}$$

where *D* is the administered dose driving the absorption process and *X* is now the amount of dose absorbed and available for excretion. The relationship between D and X is the absolute systemic availability F originally introduced in Equation 2.4 [X = FD]. In the language of differential equations, rates are simply additive, which allows the same data sets to be described in components reflecting the different processes. As above, integrating this equation and expressing it in terms of concentrations, gives the expression that describes the profile in Figure 3.9:

$$C = \frac{K_aFD}{Vd\,(K_a - K_{el})}\,[e^{-K_{el}t} - e^{-K_at}] \tag{3.24}$$

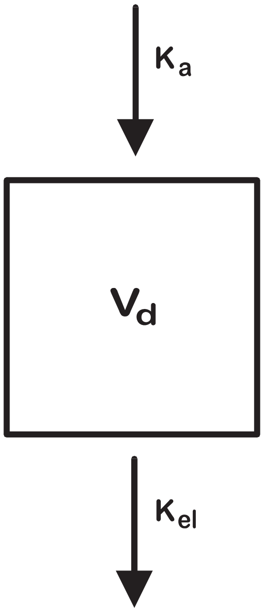

Figure 3.8 One-compartment open pharmacokinetic model with first-order absorption.

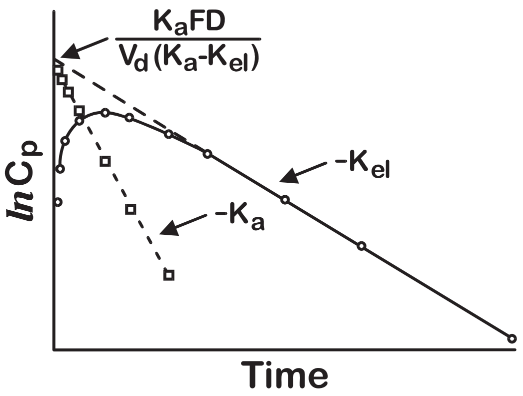

Figure 3.9 Semilogarithmic plot of plasma concentration versus time using a one-compartment open pharmacokinetic model with first-order absorption. The profile is decomposed into two lines with slopes $-K_a$ and $-K_{el}$.

This is an excellent point in the discussion to appreciate the validity of the use of multiexponential equations to describe blood C-T profiles as the exponential terms, which, like the rates above from which they were derived, are simply additive. A C-T profile is the sum of the underlying exponential terms describing the rate processes involved. This property of superposition is the basis upon which observed C-T profiles may be "dissected" to obtain the component rates. Figure 3.9 illustrates this process where an observed semilogarithmic profile is plotted as a composite of its absorption phase (controlled by K_a) and the elimination phase (controlled by K_{el}). In contrast to the intravenous scenario, the time zero intercept is now a more complex function, which is dependent upon the fraction of administered dose that is systemically available and thus able to be acted on by the elimination process described by the rate constant K_{el}. For this procedure to work, K_a must be greater than K_{el} so that at later time points e^{-Kat} approaches zero. If $K_a < K_{el}$, the same C-T profile will result; however, now the terminal slope will be K_a as it is the rate-limiting process! One just "*flip-flopped*" K_a for K_{el}. In fact, recalling the discussion in Chapter 2 on slow-release dosing formulations, we termed the resulting effect on disposition of drug in the body an example of the *flip-flop* phenomenon, the origin of which is this relation. When an extravascular route of administration is used, one can never be certain that the C-T profile is not dependent upon a slow, and thus rate-limiting, absorptive process secondary to a formulation factor. If a depot or slow-release formulation is administered such that $K_a < K_{el}$, the terminal slope will reflect the rate of absorption rather than the rate of elimination. T1/2 may be overestimated as it will now reflect $0.693/K_a$ rather than $0.693/K_{el}$. Complete absorption also cannot be assured (e.g., $F = 1$); thus one never truly knows the size of the absorbed dose. Accurate estimates of Cl and Vd, reflecting the true pharmacokinetic disposition of a drug, are required as input to determine these relations. These are best calculated after a complete intravenous injection. Finally, as discussed above for intravenous administration, urine analysis may also be used to estimate absorption parameters.

As we leave this section, it is a good time to underscore the relationship of some ADME processes as they translate to pharmacokinetic parameters; namely absorption, clearance, and volume of distribution, and further relate to the main determinants of a dosage regimen – namely, F and T1/2. This can be appreciated as

Absorption Clearance Distribution
F T1/2

F was determined by Equation 2.4 as the ratios of AUC_{oral}/AUC_{iv}. The intravenous D is a function of Cl and AUC from Equation 3.16. Half-life was a function of Vd and Cl in Equation 3.18. This scheme shows how F is a function of absorption and clearance processes (AME) while half-life is a function of clearance and distribution (DME).

Why are these relationships important? One of the main clinical applications of pharmacokinetic principles is to construct dosage regimens, the approach that is fully presented below. Diseases that change any of these primary pharmacokinetic parameters would be expected to change the plasma concentrations achieved after dosing, and thus drug effect. For example, renal disease that reduced GFR, might reduce Cl_B for drugs primarily eliminated by the kidney. Similarly, liver disease might alter disposition of drugs cleared by the liver. In contrast, diseases that resulted in severe elimination and fluid accumulation could alter Vd. Both of these scenarios would increase T1/2 and alter plasma concentrations. The relationship between concentration and effect is extensively discussed in the next chapter on pharmacodynamics.

Two-Compartment Models

Many drugs are not described by a simple one-compartment model since the plasma concentration time profile is not a straight line. This reflects the biological reality that for many drugs, the body is not a single homogeneous compartment, but instead is composed of regions that are defined by having different *rates* of drug distribution. Such a situation is reflected in the two-compartment model depicted in Figure 3.10. The drug initially is distributed in the central compartment and by definition is eliminated from this compartment. The difference comes because now the drug also distributes into other body regions at a rate that is different from that of the central compartment.

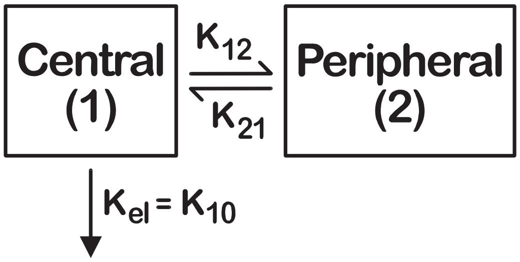

Figure 3.10 Generalized open two-compartment pharmacokinetic model after intravenous administration with elimination (K_{el}) from the central compartment. K_{12} and K_{21} represent intercompartmental micro-rate constants.

As presented in Chapter 2, there are many factors that determine the rate and extent of drug distribution into a tissue (e.g., blood flow, tissue mass, blood/tissue partition coefficient, etc.). When the composite rates of these flow and diffusion processes are significantly different than K_{el}, then the C-T profile will reflect this by assuming a biexponential nature. For many drugs, the central compartment may consist of blood plasma and the extracellular fluid of highly perfused organs such as the heart, lung, kidneys, and liver. Distribution to the remainder of the body occurs more slowly, which provides the physiological basis for a two-compartment model. Such a peripheral compartment is defined by a distribution rate constant (K_{12}) out of the central compartment and a redistribution rate constant (K_{21}) from the peripheral back into the central compartment. As discussed in the distribution chapter, depots or sinks may also occur. This is a pharmacokinetic concept where the distribution rate constants are significantly slower than K_{el} and thus become the rate-limiting factor defining the terminal slope of a biexponential C-T profile, a situation analogous to flip-flop in absorption studies.

We will begin the discussion of multicompartmental models with the principles of analyzing a two-compartment model after intravenous administration (Figure 3.11). This is the most common scenario encountered in comparative medicine and the principles easily translate to more complicated models. The fundamental principle involved is that the observed serum concentration time profile is actually the result of two separate pharmacokinetic processes that can be described by two separate exponential terms, commonly written as:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \tag{3.25}$$

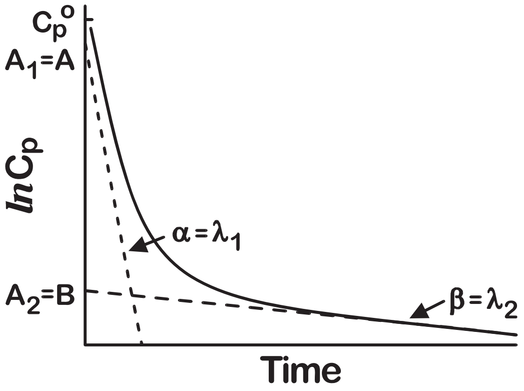

Figure 3.11 Semilogarithmic plasma concentration versus time profile of a drug described by a two-compartment open model. Parameters are defined in the text.

Note the similarity of this biexponential equation to that presented for absorption in Equation 3.24. In this case we have terms with slopes (α and β) and corresponding intercepts (*A* and *B*). The C-T profile on semilogarithmic plots is depicted in Figure 3.11. By definition, $\alpha \gg \beta$ and thus β is the terminal slope. If $\alpha \approx \beta$, the slopes of the two lines would be equal and we would be back to the single line of Figure 3.5 and a one-compartment model!

When dealing with multicompartmental models, it becomes necessary to introduce new nomenclature to denote the intercept terms and slopes of the C-T profile because, as will be shown shortly, the observed slopes are no longer synonymous with the elimination and distribution micro-rate constants as they were when we were analyzing absorption plots for K_{el} and K_a. When these models were constructed, the defining differential rate equations could be written in terms of the mass of drug in the central compartment (X). In the two-compartment model of Figure 3.10, this equation now must describe drug movement in terms of the mass of drug in compartment one and two. The solution to these differential equations are the slopes of the biexponential C-T profile giving α and β. Multicompartmental models have their own syntax: the slopes of the C-T profile are named using the Greek alphabet, starting with the most rapid rate α for distribution followed by β for elimination. The intercept terms are denoted using the Roman alphabet as A related to α and B to β.

A preferred nomenclature carries less phenomenological context and uses the Greek letter λ_n, with $n = 1, 2, 3, \ldots$ progressing from the most rapid to the slowest rate process. The corresponding intercept terms are denoted as A_n. This nomenclature describes any multicompartmental model without implying a physiological basis to the underlying mechanism responsible for the different rates observed. The biexponential equation for a two-compartment model may now be written as

$$Cp = A_1 e^{-\lambda_1 t} + A_2 e^{-\lambda_2 t} \tag{3.26}$$

The actual rate constants describing flux between compartments are now termed micro-rate constants and denoted by k_{xy}, where compound moves from $x \rightarrow y$. When the origin or destination of a compound is outside of the body, x or y is denoted as 0, respectively. K_a thus becomes k_{01} and K_{el} becomes k_{10}. With a two-compartment model, three Vds may be calculated: the volume of the central compartment V_c or V_1, the peripheral compartment V_p or V_2, and the total volume of distribution in the body V_t or $V_1 + V_2$. As will be seen below, the actual Vd calculated from the data is dependent upon the method used; however, the only estimate of V_t which can be broken into its component central and peripheral volumes is the volume of distribution at steady state, Vd_{ss}.

Now that we have the appropriate nomenclature, it is instructive to derive the differential rate equations for λ_n and A_n based on the microconstants which define them. For a two-compartment model after intravenous injection of dose D with elimination occurring from the central compartment, the following differential equation describes the rate of drug disposition:

$$dC_1/dt = -(k_{12} + k_{10})C_1 + (k_{21})C_2 \tag{3.27}$$

Processes that remove compound from the central compartment (k_{10} and k_{12}) are grouped together and have a negative rate since they result in a descending C-T profile. The only process that adds chemical to the central compartment (k_{21}) – that is, redistribution from the peripheral compartment – is assigned a positive rate and results in an ascending C-T profile. The rate of this process is driven by the concentration of compound in the peripheral compartment. Note the similarity of this equation to the differential equation for absorption in a one-compartment model (Equation 3.23). In this model, the only process that added drug to the central compartment was k_a, which therefore was assigned a positive sign, while the only process removing drug was $-K_{el}$. Similarly, as stressed throughout this text, the driving mass for this passive absorption process was the fraction of administered dose (F X) available for absorption. The power and essence of pharmacokinetic analysis is that the physiological processes driving drug disposition can be quantitated by using differential equations describing drug flux into and out of observable compartments, with most models structured to reflect the central compartment, which is monitored via blood sampling as the primary point of reference. Solution of the differential Equation 3.27 by integration yields Equations 3.25 or 3.26 describing the biexponential C-T profile characteristic of a two-compartment open model.

The observed slopes λ_1 and λ_2 and intercepts A_1 and A_2 are related to the microconstants as

$$k_{21} = (A_1\lambda_2 + A_2\lambda_1)/(A_1 + A_2) \qquad (3.28)$$

$$k_{el} = \lambda_1\lambda_2/k_{21} \qquad (3.29)$$

$$k_{12} = \lambda_1 + \lambda_2 - k_{21} - k_{10} \qquad (3.30)$$

Similarly, each of the slopes now has a corresponding T1/2 calculated as

$$T1/2\lambda_1 = 0.693/\lambda_1 \quad \{\text{Distribution}\} \qquad (3.31)$$

$$T1/2\lambda_2 = 0.693/\lambda_2 \quad \{\text{Elimination}\} \qquad (3.32)$$

The slope of the terminal phase of the C-T profile reflects the elimination T1/2 and is the primary parameter used to calculate dosage regimens. Note that since $\gamma_1 \gg \gamma_2$, $T1/2_{\gamma 1} \ll T1/2_{\gamma 2}$ and at later time points (recall the five T1/2 rule), distribution will be complete and the biexponential Equation 3.26 collapses to the monoexponential equation $Cp = A_2e^{-\lambda 2t}$. This equation is similar in form to the one-compartment Equation 3.13 except the intercept is now A_2 and not Cp_0 and the slope is $-\gamma_2$ and not K_{el}. This property of "disappearing" exponentials with large γs at later time points provides the basis for analyzing polyexponential C-T profiles using the curve "stripping" approach (technically called the method of residuals) discussed earlier.

It is often difficult to accurately estimate distribution parameters when γ_1 is very rapid since early blood samples must be collected, sometimes before blood has completely circulated. In a large animal such as a horse or cow, this requires a few minutes and thus very early samples (e.g., <5 minutes) will not have sufficient time for this mixing to occur. Secondly, small errors in sample timing result in a large % error (1 minute off for a 5-minute sample; error is 20%) and thus the data obtained at very early time points is often extremely variable. In contrast, 5 minutes off of a 6-hour sample is only a 1% error making estimates of terminal slopes much less variable.

Volumes of Distribution

There are now three volumes of distribution to contend with: V_c or V_1, V_p or V_2, and $V_t = (V_1 + V_2)$. These are again calculated by a knowledge of intercepts and administered dose (assuming intravenous administration). The relevant intercept is $C_p{}^0$, which is now simply $A_1 + A_2$:

$$V_1 = D/C_p^0 = D/(A_1 + A_2) \qquad (3.33)$$

$$Vd_{ss} = V_1[(k_{12} + k_{21})/k_{21}] \qquad (3.34)$$

$$V_2 = Vd_{SS} - V_1 \qquad (3.35)$$

$$Vd\ (B) = D/B = D/A_2 \qquad (3.36)$$

$$Vd_{area} = D/[(AUC)(\lambda_2)] = D/[(AUC)(\beta) \qquad (3.37)$$

$$= Vd_\beta = (k_{10}V_1)/\lambda_2 \qquad (3.38)$$

The relation between these estimates are

$$Vd\ (B) > Vd_{area} > Vd_{SS} > V_C \qquad (3.39)$$

The easiest to discard is *Vd(B)*, the apparent volume of distribution by extrapolation, since it is often used when a complete analysis of the curve is avoided and only the terminal slope and its intercept A_2 is determined. As discussed above, this estimate completely ignores $V_{1.}$ Similarly, V_c is defined as only the central compartment volume. It is the volume from which clearance is determined and is used in some infusion calculations.

The volume of distribution at steady-state, Vd_{ss}, is the most "robust" estimate since it is mathematically and physiologically independent of any elimination process or constant. It is the preferred Vd estimate for interspecies extrapolations and the study of the effects of altered physiology on Vd since it is independent of elimination. Theoretically, Vd_{ss} describes the Vd at only a single time point when the rate of elimination equals that of distribution. The point at which this occurs is the inflection point or bend in the C-T profile that occurs because the more rapid tissue distribution phase has now peaked. This is best appreciated in Figure 3.12 when the concentrations in the central and tissue compartments are plotted.

Vd_{area} is often used when clinical dosage regimens are constructed because it reflects the area during the elimination phase of the curve which predominates in any dosage regimen (see Figure 3.12). This is absolutely equivalent to Vd_β, the so-called volume of distribution at pseudodistribution equilibrium. If the rate of elimination is very prolonged (slow), as seen in severe renal disease, the terminal slope of the concentration-time profile may

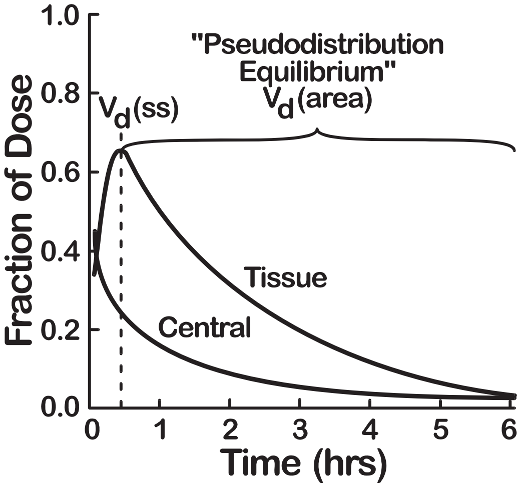

Figure 3.12 Relationship between Vd_{ss} and Vd_{area} for a drug described by a two-compartment model. Note that Vd_{ss} is only descriptive of the volume of distribution at the peak of the tissue compartment concentration versus time profile, while Vd_{area} describes the volume throughout the terminal elimination phase.

approach zero (plateaus; T1/2 becomes very long), which effectively "stretches out" the curve's inflection due to a plateau in the peripheral tissue compartment. Under this scenario, Vd_{area} becomes equal in value to Vd_{ss}.

Physiologically, a way to conceptualize Vd is to compare the individual compartment volumes based on plasma versus tissue binding, as:

$$Vd = V_{plasma} + V_{tissue}(fu_{plasma}/fu_{tissue}) \tag{3.40}$$

This relationship nicely shows the effect that both plasma and tissue protein binding can have on volume of distribution. Note that V_{plasma} and V_{tissue} do not directly correspond to V_1 and V_2, respectively, as the latter are determined by relative rates since both volumes actually include plasma and tissue.

Clearance

Knowing V_1, one can easily calculate the systemic clearance since Cl_B occurs from the central compartment and is essentially the same as a one-compartment model.

$$Cl = K_{10} V_1 \tag{3.41}$$

Alternatively, Cl_B may be calculated using the model-independent intravenous infusion Equation 3.21 presented earlier. The only difference is that with the more complex distribution kinetics present in a multicompartmental model, the time to reach C^{ss} may be significantly longer. Finally, Cl_B may also be determined using Equation 3.16 based on AUC. In a two-compartment model, AUC may be calculated using slopes and intercepts by the relation

$$\mathrm{AUC} = (A_1/\lambda_1) + (A_2/\lambda_2) \tag{3.42}$$

which can be generalized for a multicompartmental model to

$$\mathrm{AUC} = \sum A_i/\lambda_i \tag{3.43}$$

Using Vd_{ss} and Cl_B, Equation 3.18 can again be used to calculate the overall T1/2 of drug in the body. This T1/2 reflects both distribution and elimination processes and is very useful as input into an interspecies allometric analysis. This is not equivalent to the terminal elimination half-life, T1/2 (λ_2), and must be calculated from the Cl_B and Vd_{ss} parameters.

Absorption in a Two-Compartment Model

When an extravascular dose is administered as input into a two-compartment model (Figure 3.13), the differential equation defining this model is

$$V_1 dC_1/dt = -(k_{12} + k_{10})C_1 V_1 + k_{21} C_2 V_2 + k_{01} X \tag{3.44}$$

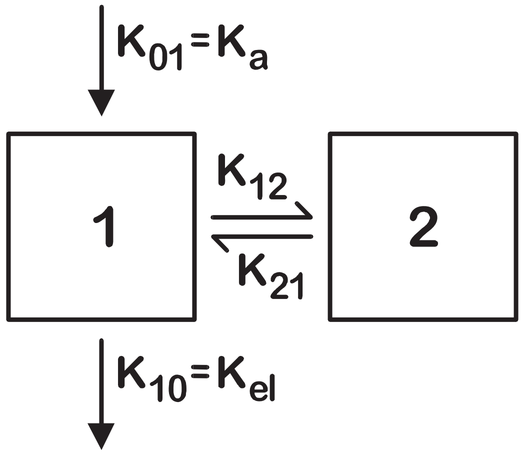

Figure 3.13 Generalized open two-compartment pharmacokinetic model with first-order absorption (K_{01}) into and elimination (K_{el}) from the central compartment. K_{12} and K_{21} represent intercompartmental constants reflecting distribution.

The movement of drug in the central compartment is now driven by three different concentrations: C_1, C_2 as well as the fraction of the administered dose D that is available for absorption (X). There are a number of approaches to solve this model. An example of the equation describing such a plasma profile would be

$$Cp = k_{01}D/V_1 \left[A_1' e^{-\lambda_1 t} + A_2' e^{-\lambda_2 t} - A_3' e^{-k_{01} t}\right] \tag{3.45}$$

In this case, the intercepts (A_n') are different than those obtained from an intravenous study (A_n) and significantly more complex since the "driving" concentrations in compartments one and two are now dependent upon the fraction absorbed in a fashion analogous to the terms of Equation 3.24 seen for absorption in a one-compartment model. However, in reality, it is difficult to separate k_{01} from λ_1 since the two are of a similar order of magnitude, coupled with the earlier discussed concern that early time points are often prone to large errors. Depending on the ratio of rate constants, the C-T profile may even appear monoexponential! The final complication is that absorption flip-flop may also occur making selection of k_{01} and λs very difficult. The only method to reliably address all of these problems is to conduct an independent intravenous bolus study using a two-compartment model and independently estimate λ_1 and λ_2 to arrive at an estimate of the absorbed dose. These equations are now easily analyzed using modern computer software.

Data Analysis and its Limitations

Clearly, as pharmacokinetic models become more complex, one must question the wisdom of pursuing such analyses. In reality, there are mathematical limitations to the complexity of the model able to be fit to an experimental data set which is based on the "information density," that is, how many data points are analyzed relative to how many parameters need to be calculated. This is similar to the statistical concept of "degrees of freedom." In practice, there are better approaches to model complex absorption using noncompartmental strategies of residence times and linear system deconvolution analysis, which are discussed in advanced texts. The final consideration with two-compartmental models, and one that is even more serious for multicompartmental models, is the actual structure of the model studied. Up until now, we have *assumed* that input into (absorption) and output from (elimination) the model are via the central compartment (model A), and furthermore, all samples are taken from this compartment and expressed as differential equations based on dC_1/dt. However, other possible structures exist for the basic two-compartment model. For example, drug may be infused into a tissue bed, or drug could distribute to the organ before metabolism and thus elimination in that organ. Many of these latter type of problems occur when the rate of distribution is actually slower than elimination making the initial exponential term reflect elimination. Very lipophilic chlorinated hydrocarbon chemicals may initially distribute extensively throughout the body and then slowly (periods of months) redistribute to the blood where metabolism would then occur. The redistribution rate constant would be the rate-limiting process. All would generate C-T profiles described by the sum of exponential very similar to those discussed above. However, the equations which *link* these fitted parameters to the underlying micro-rate constants would be very different.

Multicompartmental Models

The final level of compartmental model complexity to be dealt with in this chapter is the three-compartment model depicted in Figure 3.14 that generates the C-T profile in Figure 3.15. These data were obtained following intravenous gentamicin administration to dogs. In this case, gentamicin distributes into two different compartments from the central compartment, one with rates faster (k_{12}/k_{21}) and the other with rates slower (k_{13}/k_{31}) than k_{10}. The slopes of the C-T profile for λ_1 primarily reflects the contribution of rapid distribution while λ_3, the terminal slope, primarily reflects the contribution of slower distribution into the so-called deep compartment. This model is applicable to many three-compartment drugs encountered in veterinary medicine (e.g., aminoglycosides, tetracyclines, persistent chlorinated hydrocarbon pesticides). Drug elimination from the central compartment is primarily reflected in λ_2 or β and through general usage is termed the β *elimination phase*.

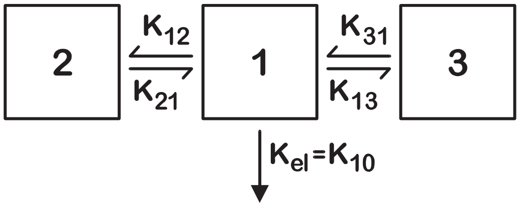

Figure 3.14 Three-compartment pharmacokinetic model after intravenous administration. Parameters are defined in the text.

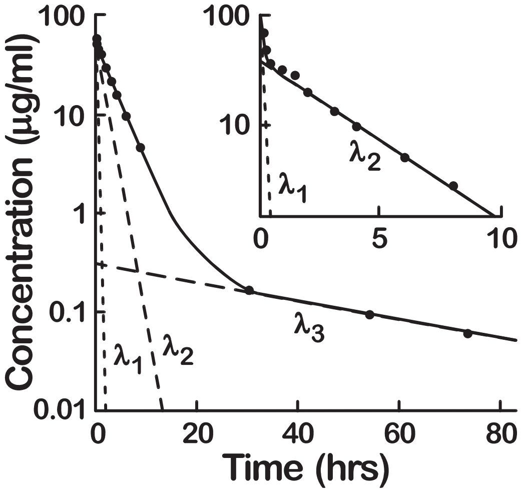

Figure 3.15 Semilogarithmic plot of plasma concentration versus time for intravenous gentamicin in the dog. Disposition is described by a three-compartment model when samples are collected over 80 hours and a two-compartment model when samples are collected for only 10 hours (insert).

These types of models are generally employed when experiments are conducted over long time frames and C-T profiles monitored to low concentrations. If the data are truncated at earlier times as shown in the insert of Figure 3.15, a normal two-compartment model is adequate to describe the data. However, if the goal of a study were to describe the tissue residue depletion profile of a drug in a food-producing animal, the tissue C_3-T profile would be of interest since it is the tissue where legal tolerances are established. This makes such complicated models useful in food animal veterinary medicine.

Models consisting of more than three compartments have been used when the data are of sufficient quality (sensitive analytical method, sufficient samples) to warrant such an analysis. The polyexponential equation describing n-compartment models (i = 1,2,3— n) is

$$C_p = \sum A_i\, e^{-\lambda_i t} \tag{3.46}$$

The differential equations needed to link these slopes and intercepts to the micro-rate constants are exceedingly complex and will not be discussed further. These complex models are only presented to give an appreciation of the types of models encountered when tissue residue predictions are encountered.

In respect to prediction of tissue residues, when a tissue sample is taken, one is not measuring just concentrations in that tissue since the vascular and extracellular fluid components of that tissue are actually part of the central compartment. Similar arguments can be made for other components. When one is looking at deep compartment disposition, this may be satisfactory since release from these depots are rate-limiting, making this tissue component larger than any other phase that has already reached equilibrium. Equations are available to fractionate a tissue mass into vascular, extracellular, and cellular components based again on Vd estimates. Alternatively, tissue cages or microdialysis probes may be inserted into the tissue mass and extracellular kinetics modeled. These data are often presented in veterinary studies dealing with antimicrobial distribution to infected tissues.

Compartmental modeling concepts and techniques have defined the discipline of pharmacokinetics and continue to be extremely useful tools. One- and two-compartment analyses form the basis for most models used in human as well as veterinary and comparative medicine. These two models also serve as the foundation upon which many of the other techniques now to be briefly discussed are based. Modern computers have facilitated the analysis of these data to the point that the user no longer has to derive all of the relevant differential equations. Comprehensive software packages are available to effortlessly perform these calculations, even if the data does not support the model analyzed! Concerns such as these have led many clinical pharmacologists in both human and veterinary medicine to move away from complex multicompartmental models and adopt so-called *model-independent* approaches when their goal is to predict dosage regimens for clinical applications.

Noncompartmental Models

Over the last two decades, there has been generalized adoption of noncompartmental methods in veterinary and comparative pharmacokinetics. Noncompartmental models were first developed and applied to radiation decay analysis and remain dominant in the physical and biological science literature for general applications. Since their first application to problems in pharmacokinetics by Yamaoka in 1979, noncompartmental methods have grown steadily in use. This approach is for the most part actually an application of well-developed statistical moment theory, a full discussion of which is beyond the scope of this text. The approach involves primarily calculation of the Slopes, Heights, Areas and Moments (SHAM) of plasma concentration time curves. Statistical moment theory describes drug behavior based on the mean or average time an administered drug molecule spends in a kinetically homogeneous space, a concept identical to that of a compartment. The difference again is that no specific inferences are being made about the structure of these spaces.

Rather than being based on diffusion, these models are based on *probability density functions* that define drug disposition in terms of the probability of the drug being in a specific location. Instead of determining rates in terms of rate constants or half-lives, they describe processes in terms of statistical moments; the most useful is the mean residence time (MRT; τ). These are based on plasma concentration data and are determined by calculating areas under concentration versus time curves. MRT is calculated as

$$MRT = \frac{\int_0^\infty t\,C\,(t)\,dt}{\int_0^\infty C\,(t)\,dt} = \frac{AUMC}{AUC} \tag{3.47}$$

The denominator of this equation is the AUC we have discussed earlier in terms of calculating clearance and bioavailability. The numerator is known as the area under the [first] moment curve (AUMC), which is the CT-T profile. AUC and AUMC are depicted in Figure 3.16.

The MRT could be thought of as the statistical moment analogy to the half-life (T1/2), and it is inversely related to the first-order elimination rate of a one-compartment open model:

$$MRT_{TV} = 1/K_{el} \tag{3.48}$$

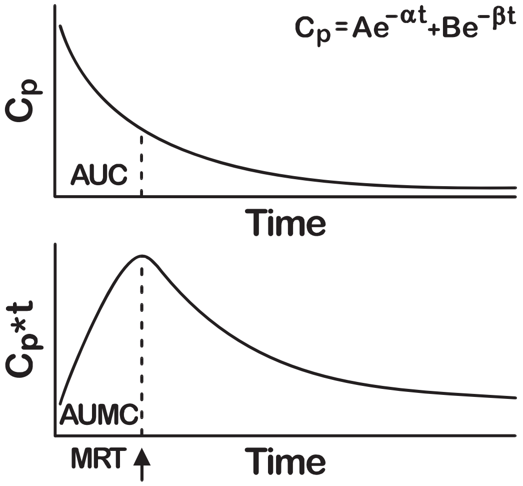

Figure 3.16 Plasma concentration versus time (C-T) and its first-moment (CT-T) plots demonstrating AUC, AUMC, and MRT.

Rearranging demonstrates that $K_{el} = 1 / MRT_{iv}$. Recalling Equation 3.18 where T1/2 = 0.693/K_{el}, substitution gives us

$$T1/2 = 0.693\ (MRT) \tag{3.49}$$

The MRT thus becomes an excellent parameter to describe the length of drug persistence in the body, much as the half-life is used in many compartmental pharmacokinetic models. The T1/2 used in this context is the elimination T1/2 in the body, and not that calculated from the terminal exponential phase for multicompartmental models. If the dose of drug is administered by intravenous infusion, the MRT_{iv} may be calculated as

$$MRT_{iv} = MRT_{infusion} - (Infusion\ Time)/2 \tag{3.50}$$

where $MRT_{infusion}$ is simply calculated from the observed data using Equation 3.47.

The primary task to solve model–independent or noncompartmental models is the direct estimation of the moments from data. This essentially is determining the relevant AUCs and moments from the C-T profile. When the C-T profile is described by a polyexponential equation of the form $f(t) = A_i e^{-\lambda it}$, Equation 3.43 ($AUC = \Sigma A_i / \lambda_i$) can be generally used to determine AUC. The AUMC may then be calculated as

$$AUMC = \sum A_i/\lambda_i^2 \tag{3.51}$$

The simplest and most commonly used method for estimating area under any curve is the trapezoidal rule. This technique is important since it is the primary method used to assess bioavailability by regulatory agencies. The approach is again illustrated in Figure 3.17.

$$AUC = \sum_{n=1}^{N} \frac{C_n + C_{n+1}}{2}(t_{n+1} - t_n) \tag{3.52}$$

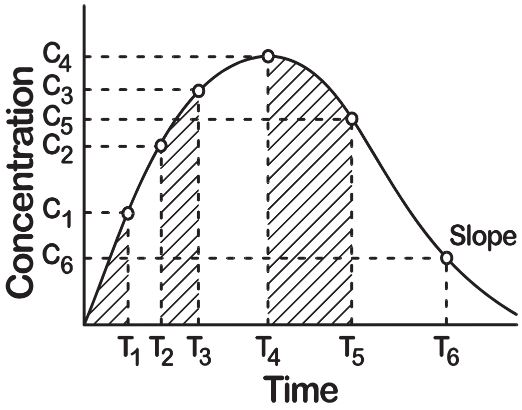

Figure 3.17 Breakdown of a plasma concentration versus time curve into trapezoids used to calculate the area under the curve. The terminal area from T_6 to T_∞ is calculated from extrapolating the terminal slope.

The summation is over N trapezoids, formed by N+1 data points. This algorithm is quick and, if enough data points are available, relatively accurate. It is also a simple algorithm to implement on a computer. The area under each pair of connected points describes a trapezoid (except when one of the points has zero value, in which case one of the legs of the trapezoid has zero length, making a triangle). The area under the entire curve is then the sum of the areas of the individual trapezoids, which can easily be calculated. The area under the final triangle is estimated by the AUC and must be estimated to infinite time. Generally, this portion of the AUC should be less than 20% of the total. Many methods have been proposed for this, the most common being $AUC_{T\to\infty} = C_T / \lambda_n$, where λ_n is the terminal slope of the C-T profile. The estimation of the first moment for calculation of MRT is the summed trapezoids plotted on a CT-T graph. The attraction of statistical moment analysis to pharmacokinetics is the use of trapezoids to determine the relevant areas. No assumptions but the underlying mechanisms of drug disposition are made, and computer curve fitting is not required.

To complete this brief introduction to this topic that is the basis of many commercial software packages, one other residence time having general application in clinical pharmacology is the mean absorption time (MAT). Other residence times may be calculated but are not used in determining dosage regimens in clinical medicine. MAT is technically the mean arrival time into the systemic circulation of bioavailable absorbed molecules. MAT is the statistical moment theory equivalent of estimating K_a. MAT is a computationally straightforward method to characterize the rate of drug absorption in bioavailability studies. The simplicity of this approach is that transit times are additive. MAT is the mean time for drug molecules to remain unabsorbed. MAT is simply the difference in MRT following intravenous injection (MRT_{IV}) and another noninstantaneous administration (MRT_{Route}):

$$MAT = MRT_{Route} - MRT_{IV} \tag{3.53}$$

Assuming absorption is described by a first-order process with an apparent rate constant of k_a, then

$$MAT = k_a^{-1} \tag{3.54}$$

making the absorption half-life

$$T1/2_{[abs]} = \ln 2 \cdot MAT \tag{3.55}$$

On the other hand, when absorption is assumed to be a zero-order process (e.g., constant rate), then

$$MAT = T/2 \tag{3.56}$$

where T is the duration of the absorption. Note the similarity to the infusion Equation 3.50 above. In reality, a constant rate infusion is a zero-order absorption whose MAT is just one-half the length of the infusion.

The reader should recall that the determination of systemic availability expressed in Equation 2.4 [$F = (AUC_{Route})(Dose_{IV})/(AUC_{IV})(Dose_{Route})$] is a noncompartmental analysis. An AUC should be determined by the trapezoidal methods presented in this chapter. If the point of an analysis is to determine bioequivalence between two formulations, one is actually calculating relative systemic availability, a concept equivalent to determining whether two formulations are clinically interchangeable. In addition, the metrics time to peak concentration (T^{max}) and peak concentration (C^{max}) are often compared. Comparison of MATs would also shed light on the equivalence of two formulations. The reader should consult the references in the section "Selected Readings," later in this chapter for further approaches and the Food and Drug Administration website (www.fda.gov/CVM) for current bioequivalence guidelines for veterinary products.

The determination of Cl_B using statistical moment theory is easily obtained using the previously defined Equation 3.16 where $Cl_B = D/AUC$. Using the trapezoidal methods to estimate AUC makes this a robust estimate of clearance. The volume of distribution at steady-state (Vd_{ss}), according to statistical moment theory, is simply the product of MRT and CL:

$$Vd_{SS} = Cl_B\ MRT \tag{3.57}$$

This, incidentally, affords the expression for half-life as a function of clearance, by solving Equation 3.57 for MRT:

$$T_{1/2} = \frac{\ln 2 \cdot Vd_{SS}}{Cl_B} \tag{3.58}$$

which is the same as that presented in Equation 3.18. Substitution of the respective expressions for MRT (Equation 3.47) and CL (Equation 3.16) into Equation 3.57 yields

$$Vd_{SS} = \frac{D_{i.v.} \cdot AUMC}{AUC^2} \tag{3.59}$$

Another volume parameter also calculated using statistical moments sometimes encountered for dosage regimens is Vd_{area}:

$$Vd_{area} = \frac{D_{i.v.}}{k_{el} \cdot AUC} \tag{3.60}$$

Statistical moment methods provides a powerful tool for calculating many of the common pharmacokinetic parameters that are routinely encountered in veterinary medicine. This includes the concept of bioequivalance discussed above, as well as generating parameters that are used to construct dosage regimens and assess the effect of disease on drug effects. As can now be even more fully appreciated, Cl_B and Vd_{ss} are truly independent parameters that quantitate distribution and excretion using computationally robust techniques based on minimal model-specific assumptions. We have presented this approach since it is the primary method by which pharmacokinetic parameters are now determined in veterinary medicine. Table 3.2 is a compilation of equations useful to calculate these parameters from an analysis of a C-T profile.

Table 3.2 Noncompartmental equations for calculating common pharmacokinetic parameters

$Cl_B = Dose / AUC$
$Cl_D = V_c\ \lambda_1 - Cl_B$
$Vd_{ss} = (Dose \times AUMC) / AUC^2$
$V_c = Dose / Cp_0$
$MRT_{iv} = AUMC/AUC = V_d(ss) / Cl_B$
$MAT = MRT_{route} - MRT_{iv}$
$T1/2 = 0.693\ MRT = 0.693\ Cl_B / Vd_{ss}$
$T1/2\ (\lambda) = 0.693 / \lambda$
$F = (AUC_{route})\ (Dose_{iv}) / (AUC_{iv})\ (Dose_{route})$
$AUC = \Sigma\ A_i / \lambda_i$
$AUMC = \Sigma\ A_i / (\lambda_I)^2$

Note that AUC and AUMC could be calculated using trapezoidal analysis of areas rather than fitting curves to obtain estimates of A_i and λ_i.

Nonlinear Models

Most pharmacokinetic models incorporate the common assumption that drug elimination from the body is a first-order process, and the rate constant for elimination is assumed to be a true constant, independent of drug concentration. In such cases, the amount of drug cleared from the body per unit time is directly dose or concentration dependent, the percentage of body drug load that is cleared per unit time is constant, and the drug has a single constant elimination half-life. Fortunately, first-order elimination (at least apparent first-order elimination) is typical in drug studies. First-order linear systems application greatly simplifies dosage design, bioavailability assessment, dose–response relationships, prediction of drug distribution and disposition, and virtually all quantitative aspects of pharmacokinetic simulation.

However, drugs most often are *not* eliminated from the body by mechanisms that are truly first-order by nature. Actual first-order elimination across all concentrations applies only to compounds that are eliminated exclusively by mechanisms not involving enzymatic or active transport processes (i.e., processes involving energy). As presented in Chapter 2, they are primarily driven by diffusion and obey Fick's Law. The subset of drugs not requiring a transfer of energy in their elimination is restricted to those that are cleared from the body by urinary and biliary excretion and, among those, only drugs that enter the renal tubules by glomerular filtration or passive tubular diffusion. All other important elimination processes require some form of energy-consumptive metabolic activity or transport mechanism. What is the impact of this on pharmacokinetic parameters?

The reason energy-involved processes are not strictly first-order is that they are generally *saturable,* or more specifically are *capacity-limited.* At clinical dosages, the majority of drugs do not reach saturation concentrations at the reaction sites, and follow first-order linear kinetics. Recalling for first-order processes, a constant percentage of remaining drug is cleared per unit time, and the drug has a discrete, concentration-independent elimination rate constant (K) and thus half-life. For drugs eliminated by zero-order kinetics or saturated pathways, however, a constant quantity of drug is eliminated per unit of time, and this quantity is drug concentration independent and the drug does not have a constant, characteristic elimination half-life. The potential impact of saturable, leading to zero-order (versus first-order) elimination, can be profound, and its effects include altered drug concentration profiles, scope and duration of drug activity, and distribution and disposition among tissues. Saturable hepatic metabolism may markedly affect drug absorption due to reduced clearance (lower hepatic extraction) and altered first-pass activity after oral administration. Nonlinearity is associated with a nonconstant T1/2 at different doses or when a plot of dose versus AUC is not linear, indicating that Cl is reduced as dose increases.

The primary technique used to model saturable metabolic process employs the Michaelis–Menten rate law. This can be expressed as

$$dC/dt = -(V_{\max})(C)/(K_m + C) \tag{3.61}$$

where V_{max} is the maximum velocity (rate) of the reaction, and K_m is the Michaelis constant that relates concentration to effect. There are two notable simplifying conditions of the Michaelis–Menten equation. If $K_m \gg C$, then Equation 3.61 reduces to

$$dC/dt = -(V_{\max})(C)/(K_m) \tag{3.62}$$

This is equivalent to first-order elimination after IV administration in a one-compartment model where $dC/dt = -K_{el}C$. Thus, assuming elimination by a single biotransformation process, the first-order elimination rate constant K_{el} becomes V_{max}/K_m. If however, $K_m \gg C$, saturation is occurring and then Equation 3.62 collapses to

$$dC/dt = -V_{\max} = -K_0 \tag{3.63}$$

The rate in this case is independent of drug concentration (i.e., a constant), which describes a zero-order process, and the rate of drug elimination is now equal to $-K_0$.

Often, drugs are found to be eliminated by both first-order and nonlinear processes in parallel. In such cases, Equation 3.62 must be expanded to include the strictly first-order elimination processes:

$$\frac{dC}{dt} = -\frac{V_{\max}C}{K_m + C} - k'_{el}C \tag{3.64}$$

What is the impact of this on clinical veterinary pharmacology? The precise calculation of V_{max} and K_m is not done in clinical practice. However, values of Cl_B and T1/2 are assumed to be constant. When saturation occurs, Cl_B decreases and T1/2 increases, resulting in dosage regimens that accumulate with potential adverse effects. Recalling discussions in Chapter 2 on metabolism and saturation, the same phenomenon can also occur if enzyme inhibition occurs. In contrast, enzyme induction would increase clearance and reduce T1/2 resulting in decreased and potentially ineffective plasma concentrations.

Summary of Modeling Approaches

With the advent of modern computers, advanced software packages and sensitive and high-throughput analytical techniques, more comparative pharmacokinetic data are becoming available, and such data are being included in package inserts of approved products. The focus of this chapter is to develop basic pharmacokinetic concepts that serve as the foundation of dosage regimen construction in clinical practice. Obviously, many of the more complex models were only presented in order to illustrate these basic concepts. It is crucial that the practitioner know assumptions and limitations involved in a specific clinical case. However, there are additional pharmacokinetic approaches that are used in comparative medicine which in some cases complement the basic approaches above, and in others offer significant advantages. Two of these will be briefly introduced. Standard pharmacokinetic textbooks should be consulted for more detail.

Population Pharmacokinetics

All of the models discussed to this point have been focused on predicting drug concentrations in the individual animal. However, in many cases populations are of interest. For example in dogs, it would be ideal to know the basic pharmacokinetic parameters for a drug in the population at large that would apply to all breeds, ages, and gender. More important would be knowledge of which subpopulation had significantly different parameters. This is normally achieved by collecting a large number of plasma samples in individual animals and averaging resulting pharmacokinetic data from small (4–6 animals) studies. Recently, techniques have been developed that allow one to conduct studies in large numbers of individuals with less individual sampling. The approach uses very simple pharmacokinetic models (e.g., Equation 3.13) or SHAM approaches, and collect more physiological data (body weight, age, creatinine) to solve the models. For example, instead of estimating Cl_B only from C-T data, one establishes a relation between GFR and Cl_B, and through statistical approaches, uses both data sets. The mathematics and statistical approaches in these so-called "mixed-effect" models (called *mixed* because kinetic and statistical models are combined) are beyond the scope of the present text. However, their adoption by scientists in drug development areas coupled with commercial software platforms that facilitates their use will ensure that better estimates of pharmacokinetic parameters, applicable to populations, will be available.

This is especially important in veterinary medicine where breed and species differences are extreme and data quantitating these differences are scarce. Recently, many additional so-called population pharmacokinetic studies have been conducted in veterinary medicine as a tool to combine pharmacokinetic data across different studies when specific endpoints are desired. They are not used in the same manner as they are in large, well-funded phased human clinical trials; however, they provide a tool for combining datasets across disparate published studies and smaller clinical trials. For example, data from field studies have been combined with regulatory datasets to predict tissue withdrawal times after drugs such as flunixin have been administered to disease animals requiring a prolonged withdrawal time before slaughter (see Wu et al., 2013; Li et al., 2014).

Physiological-Based Pharmacokinetic (PBPK) Modeling

The second approach to studying pharmacokinetics in animals and humans is PBPK modeling. This approach is fundamentally different than discussed above as models are constructed by defining the body as a series of anatomical organs and tissues connected by the vascular system (Figure 3.18). Data is collected in plasma as well as tissue compartments, which are defined both on the basis of overall effect on drug disposition as well as sites of action or toxicity. Data input consists of blood flows into tissues as well as the partitioning coefficients for drug between blood and tissues. The model is solved in terms of a series of mass-balance equations defining input and output from each organ, very similar to that discussed when clearance was introduced in the last chapter (Equation 2.6). Advanced software packages facilitate solving these complex equations.

These models are extensively employed in the field of toxicology where data collected in laboratory animals are used to extrapolate to humans. In these cases, models may be defined in mice or rats, and then human physiological parameters inputted to estimate human disposition. The models are well suited to integrate in vitro laboratory data on toxicity or effect, as well as being able to simultaneously model parent drug and metabolite disposition. They would be ideal to model the so-called pharmacodynamic effect compartment introduced in Chapter 4. They have recently been used to study tissue residue depletion in food animals because they allow for predictions in the target tissues monitored by veterinary regulatory authorities. Similar to the ability of population models to analyze data across diverse sets of data, PBPK models can do the same in addition to detailing more physiologically realistic absorption models and incorporating in vitro data (see recent examples by Leavens et al., 2014; Lin et al., 2015). A comprehensive review detailing how PBPK models work and applications to veterinary medicine has recently been published (Lin et al., 2016).

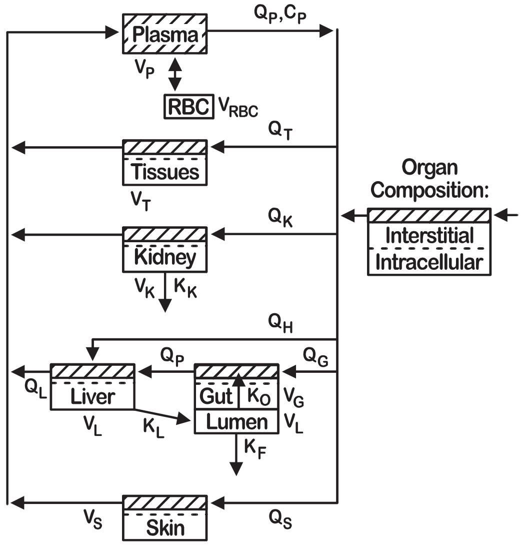

Figure 3.18 Structure of a physiologically based pharmacokinetic model (PBPK) incorporating disposition in plasma, liver, kidney, skin, and the gastrointestinal tract. Elimination from the body occurs from the kidney (K_K), liver (K_L), and gut lumen in feces (K_F). Oral absorption is allowed (K_O). V refers to organ volumes of distribution and Q to organ blood flows.

Dosage Regimens

The primary use of pharmacokinetics in a clinical setting is to calculate safe and effective drug dosage regimens for patients. These are generally based on target plasma drug concentrations that are believed to be therapeutically effective. The dose required to achieve and then maintain these target concentrations must be calculated using a knowledge of the drug's pharmacokinetic parameters in these individuals.

This concept is best addressed by visualizing the drug's C-T profile after multiple dose administration as depicted in Figure 3.19. There are two descriptors of the dosage regimen that are important to describe a

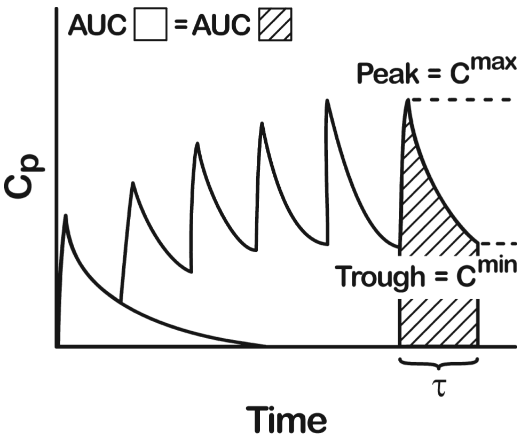

Figure 3.19 Plasma concentration (Cp) versus time profile after multiple extravascular drug administration demonstrating accumulation. Peak and trough concentrations represent those after achievement of steady-state where the AUC under a dosing interval τ (hatched area) is equal to that after a single dose (unshaded area).

multiple dose regimen. These are the dose and dosage interval (τ). The dose is further classified as the initial or *loading dose* (D_L) required to rapidly achieve an effective plasma concentration and the *maintenance dose* (D_M) needed to sustain these concentrations. The resulting profile is characterized by *peak* (C^{max}) and *trough* (C^{min}) plasma concentrations, which result after the animal has achieved a steady-state condition.

The shape of such a multiple dosage regimen is dependent upon the relationship between the T1/2 of the drug and the length of the dosage interval, τ. Assuming that a single dose of drug is administered to an animal, the resulting C-T profile after extravascular administration will resemble that depicted in Figure 3.7 (plotted on a semilog C-T axis), which (plotted on a normal C-T axis) is the left-most profile in Figures 3.19 and 3.20. The AUC of this C-T segment describes the quantity of drug cleared from the body (Equation 3.16). If a second dose of drug were given after the first dose were completely eliminated (e.g., approximately five T1/2s), then this profile would be repeated as depicted in Figure 3.20. This dosage regimen, where τ is ≫ 5 T1/2s, does not result in any drug accumulation in the body. The peak and trough plasma concentrations of this multiple dose regimen is the same as seen after a single dose and the equations presented earlier (Equation 3.13 for an intravenous dose and Equation 3.24 for an extravascular dose) may be used to describe the profile. The dose required to achieve a specific peak concentration or C^{max} after administering a very rapidly absorbed preparation is essentially obtained by rearranging the equation for volume of distribution (Equation 3.12) which becomes

$$Dose = (C^{\max})\, Vd \tag{3.65}$$

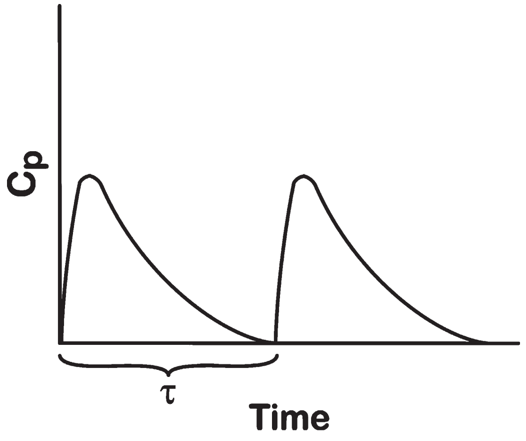

Figure 3.20 Plasma concentration (Cp) versus time profile after multiple extravascular drug administration with no accumulation, resulting in independent pharmacokinetics described by two single-dose profiles.

If the drug is not completely bioavailable (e.g., $F < 1$), this equation is divided by the systemic availability F. Administering this dose at every τ will result in the same C-T profile characterized by C^{max} and a C^{min} of essentially zero.

However, the more likely scenario is that depicted in Figure 3.19 where a second dose is administered before the first dose is completely eliminated from the body. In this case, the drug concentrations will accumulate with continued dosing. This accumulation will stop or reach a *steady-state* when the amount of drug administered at the start of each dosing interval is equal to the amount eliminated during that interval. This can be appreciated since the AUC under one dosing interval is equal to that after a single dose administration. In fact, steady-state could be defined as the dosing interval where the AUC for that interval is equal to the single dose AUC. Administering repeated doses at a τ defined in this manner will continuously produce a C-T profile with the same peak and trough plasma concentrations.

There are a series of simple formulae derived from basic pharmacokinetic principles that can be used to precisely derive these profiles. The first is to determine C^{avg}, which is a function of the Vd and the ratio of the T1/2 and τ where

$$C^{avg} = \frac{(1.44)(F)(D)}{Vd_{area}} \bullet \frac{T1/2}{\tau} = \frac{C^{\max} - C^{\min}}{\ln(C^{\max}/C^{\min})} \tag{3.66}$$

Recalling the relationship between T1/2, Cl and Vd presented earlier in Equation 3.18, rearrangement yields $1/Cl = 1.44\ T1/2/Vd_{area}$. It is instructive when studying these relations to recall that 0.693 is the ln 2 and 1.44 is 1/ln2. Substituting this into Equation 3.66 yields

$$C^{avg} = (F/Cl)\ (D/\tau) \tag{3.67}$$

Another relationship becomes evident from these formulae. We learned earlier that Cl is defined as D/AUC, which allows Equation 3.67 to be algebraically expressed as $C^{avg} = (F)\ (AUC)/\tau$. This relation quantitates the observation presented earlier that the AUC under any dosing interval τ will always be the same when steady-state conditions are achieved.

Two factors that are important in designing dosage regimens emerge from Equations 3.66 and 3.67. The ratio D/τ can be defined as the *dosage rate* and is the major determinant under the control of the clinician that determines the amount of drug which will accumulate in the body. The ratio $T1/2/\tau$ is a proportion relating the relative length of the dosing interval to the half-life of the drug. If the inverse of this ratio is taken, the *relative dosage interval* ε may be defined as: $\varepsilon = \tau/T1/2$. These two ratios provide useful parameters to gauge the shape and height of the C-T profile produced by a tailored dosage regimen, as well as adjust dosages in disease states with reduced clearances.

The primary factor governing the extent of drug accumulation in a multiple dose regimen is the fraction of a dose eliminated in one dosing interval, termed f_{el}. This can be easily calculated by first determining the amount of drug remaining at the end of a dosing interval, f_r. We showed earlier that the amount of drug in the body at any time t is given by the exponential (Equation 3.6) relation $X_t = X_0\ e^{-kt}$. If one substitutes τ for t at the end of a dosing interval, then f_r is defined as

$$f_r = 1 - f_{el} = X_t/X_0 = e^{-k\tau} = e^{-\lambda\tau} \tag{3.68}$$

where k is the fractional elimination constant or the relevant slope of the terminal phase of the C-T profile governing the disposition of the drug. It can be rearranged to

$$f_{el} = 1 - e^{-0.693(\tau/T1/2)} = 1 - e^{-0.693\ \varepsilon} \tag{3.69}$$

Using f_{el}, the peak and trough concentrations may be calculated as

$$C^{max} \frac{(F)\ (D)}{(Vd_{area})\ (f_{el})} \tag{3.70}$$

$$C^{min} = (C^{max})\ (1 - f_{el}) \tag{3.71}$$

The ratio of the fluctuation in $C_p{}^{max}$ and $C_p{}^{min}$ can be determined as

$$C^{max}/C^{min} = e^{0.693(\tau/T1/2)} = e^{0.693\ \varepsilon} \tag{3.72}$$

and further,

$$\ln\ (C^{max}/C^{min}) = 0.693(\tau/T1/2) = 0.693\ \varepsilon \tag{3.73}$$

This is an extremely powerful relationship that demonstrates that the magnitude of fluctuations in a C-T profile is directly related to the *relative* dosage interval ε. As either τ increases or $T1/2$ decreases, this ratio will get larger and result in a greater fluctuation in drug concentrations.

Using these relationships and rearranging Equation 3.70 to solve for dose, one can now derive the dosage formulae required to achieve a C-T profile with specified target peak and trough concentrations:

$$\begin{aligned} D_M &= (f_{el})\ (Vd_{area})\ (C^{max})/F \\ &= Vd_{area}(C^{max} - C^{min})/F \end{aligned} \tag{3.74}$$

$$\begin{aligned} D_L &= (D_M)\ (f_{el}) \\ &= (Vd_{area})\ (C^{max})/F \end{aligned} \tag{3.75}$$

Thus to construct a dosage regimen, the only parameters that are required are the Vd_{area} and $T1/2$ (and hence λ or k_{el}) of the drug to calculate f_{el}. If one is targeting only the average plasma concentration, then Equation 3.66 can be solved for dose as

$$\begin{aligned} D_M = \frac{(C^{avg})(Vd_{area})}{(1.44)(F)} \times \frac{\tau}{T1/2} \times \frac{(0.693)\ (C^{avg})\ (Vd_{area})}{F} \\ \times \frac{\tau}{T1/2} \times \frac{(C^{avg})(Cl)(\tau)}{F} \end{aligned} \tag{3.76}$$

The magnitude of f_{el} is a good estimate of the degree of fluctuation that occurs between the peak and trough concentration at steady-state. As the ratio of $\tau/T1/2$ or ε approaches zero, f_{el} approaches zero and the amount of fluctuation in a dosage interval is minimal. In contrast, when this ratio is large, f_{el} approaches one and the peak and trough concentrations have a large degree of fluctuation.

When f_{el} is very small, C^{max} approaches C^{min} since f_{el} approaches zero when τ approaches zero. The resulting C-T profile becomes characterized by C^{avg}. This occurs when the drug is given as an intravenous infusion. The rate of an intravenous infusion (R mg/min) is essentially an *instantaneous* dose rate and is equivalent to D/τ. When R is inserted in Equation 3.67 for D/τ and F is set equal to 1 (since an intravenous dose is be definition 100% systemically available),

$$C^{avg} = R/Cl \tag{3.77}$$

and

$$R\ (mg/min) = (C^{avg})\ (Cl) \tag{3.78}$$

which is identical to Equation 3.20 presented earlier for the steady-state plasma concentration (C^{ss}) obtained after administering an intravenous infusion. If one desires that concentration be achieved immediately, then a loading dose may be calculated as

$$D_L = (C^{avg})\ (Vd) \tag{3.79}$$

Comparing Equations 3.66 and 3.77 is instructive since it demonstrates the dependence of the average steady-state concentration on the rate of drug input (R or D/τ) in mass/time and the rate of drug elimination exemplified by the clearance. When constructing and comparing dosage regimens, the C^{avg} of any two regimens will always be the same as long as D/τ is constant. This factor becomes very important when dosage regimens are constructed for patients with impaired clearance due to renal disease. The larger the f_{el} or ε is in a single dosage interval τ, the greater the fluctuation in plasma concentrations. To minimize fluctuation but maintain a constant C^{avg}, smaller maintenance doses (D_M) must be administered at shorter dosage intervals.

It is also important to stress at this point that the length of time required to achieve steady-state for any dosage regimen is solely based on the rate-controlling λ or T1/2 of the drug in the clinical situation. Thus with drugs having a prolonged half-life, a loading dose may often have to be administered to rapidly achieve therapeutically effective plasma concentrations.

The principles presented in this chapter may be widely applied for a number of therapeutic situations. For most drugs employed in a clinical setting, the relevant T1/2 will be that governing the elimination of the drug's C-T profile. If the drug exhibits nonlinear pharmacokinetic properties and accumulates, saturation may occur and the clearance operative at low concentrations may be reduced at the higher concentrations seen at steady-state, leading to further accumulation and potential toxicity. In contrast, if the drug induces its own metabolism, the disposition and thus required dosage regimen may be greater after chronic use to prevent relapse. This has been seen with certain anticonvulsant drugs.

Other considerations are operative when administering extravascular drugs with relatively slow rates of absorption. In many cases, the governing rate process may be the absorption phase (k_a) and a flip-flop situation will occur. In this case, it will take five *absorption* T1/2s to reach steady-state. These approaches to constructing dosage regimens are ideally suited when pharmacokinetic parameters are obtained from the noncompartmental models. In these cases, the same equations may be used.

Comments on Efficacy and Safety

The final point to consider is the relationship between the target C^{max} and C^{min} concentrations and therapeutic or toxic effect. This relation is best presented in Figure 3.21, which depicts a hypothetical C-T profile versus various target Cs for efficacy and toxicity. The precise manner by which a pharmacokinetic profile is linked to its pharmacodynamic or toxicodynamic effects will be presented in Chapter 4. This relationship is very dependent upon the pharmacology of the drug itself, and cannot be easily generalized across all drugs. As will be discussed in the next chapter, drug potency, efficacy, sensitivity, and specificity define the mathematical nature of the link between the C-T profile and effect. Individual chapters of this text must be consulted for an overview of mechanism of drug action, that is, what biochemical receptors are targeted by the individual drug. If an antimicrobial is involved, the mechanism of bacteria killing is important.

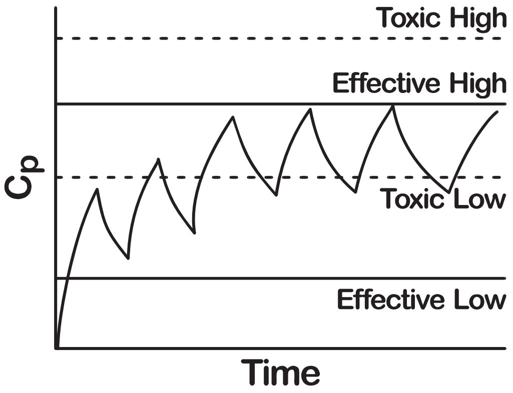

Figure 3.21 Relationship between a multiple-dose plasma concentration versus time profile and thresholds for efficacy and toxicity, which define the therapeutic benefit/risk ratio for the drug.

It is instructive to realize that the form of a C-T profile is by itself only dependent upon the dosage regimen parameters (D/τ) and the pharmacokinetics of the drug in the patient. The resulting efficacy or toxicity of the profile is dependent upon the underlying pharmacology and toxicology of the drug. These biological effects are often, though not always, correlated to plasma concentrations. Thus, for a specific drug, one might have data that would indicate that efficacy would occur when C is maintained above the *effective low* concentration and toxicity would be expected to occur only if concentration exceeded the *toxic high* level plotted. In this case, the dosage regimen that produced this C-T profile would be considered optimal since at steady-state it is well within the *therapeutic window* defined by these two thresholds. However, if the efficacy of the drug were defined by the *effective high* threshold plotted, the regimen would not be therapeutically effective. Similarly, if the relevant toxicological threshold were the *toxic low* line in this figure, one would predict that this regimen would be unsafe to administer.

Adjusting for Disease

One of the primary and most common factors that affects the disposition of a drug in the body is disease-induced changes in renal function. It is no surprise that renal disease has a profound impact on the disposition of a drug in the clinical setting. Many drugs are excreted primarily in urine as unchanged pharmacologically active drug. Drugs excreted in this manner accumulate in the body during renal insufficiency as a direct result of decreased renal clearance and is the primary manifestation of renal disease, which is compensated for in clinical dosage adjustment regimens. Renal disease can also influence other aspects of drug disposition, including altered protein binding, volume of distribution, and hepatic biotransformation. All of these effects complicate the establishment of safe and efficacious regimens for drug therapy. The chapters on specific drugs should be consulted when considering their use in such patients.

Of course, the obvious choice for selecting a drug for a patient with renal disease is to use a drug not cleared by the kidney. If this is not possible, current approaches for constructing adjusted dosage regimens for renal insufficiency or failure compensate only for decreased renal clearance of the parent drug and are based upon the principles of dosage regimen construction discussed above. In this approach, we assume (1) a standard loading dose is administered; (2) drug absorption, volume of distribution, protein binding, nonrenal elimination, and tissue sensitivity (dose–response relation) are unchanged; (3) creatinine clearance is directly correlated to drug clearance; and (4) there is a relatively constant renal function over time.

The ultimate aim of dosage adjustment in renal disease is to fulfill the fundamental therapeutic postulate that the C-T profile should be as similar as possible to the normal situation. Recall above that ε, the ratio τ/T1/2, determines the fluctuation in a multiple dose C-T profile based on its influence on the value of f_{el} from Equation 3.69. The dose ratio, D/τ, determines the average steady-state plasma concentration (C^{avg}). If τ is not adjusted in the face of an increasing T1/2 in a patient with renal failure, C^{avg} will dramatically increase, as can be seen from revisiting Equation 3.66. This can be compensated either by reducing D or increasing τ in this equation, which is the basis of the dose modification methods introduced below. However, the fluctuations in these regimens is a function of f_{el} which in a renal disease patient is dependent upon ε. Thus τ must be increased to compensate for the prolonged T1/2 if f_{el} and thus fluctuations are to be dampened. When constructing dosage regimens for patients in renal disease that has fundamentally altered pharmacokinetic parameters, both D and τ must be modified to achieve C^{avg} and f_{el}, which are similar to the patient with normal renal function.

Let us assume that one has already defined a safe and effective dosage regimen for use in a normal patient. These normal dosage regimens are then adjusted according to the dose fraction by two basic procedures. The first method, termed *constant-interval, dose-reduction* (DR), reduces the dose (D) by a factor of the dose fraction. Dose interval (τ) is the same as that used in the healthy animal. The dose fraction K_f is calculated as the ratio of diseased/healthy drug Cl, GFR or creatinine clearance.

$$\begin{aligned} D_{renal\ failure} &= D_{normal}\,K_f \\ \tau_{renal\ failure} &= \tau_{normal} \end{aligned} \tag{3.80}$$

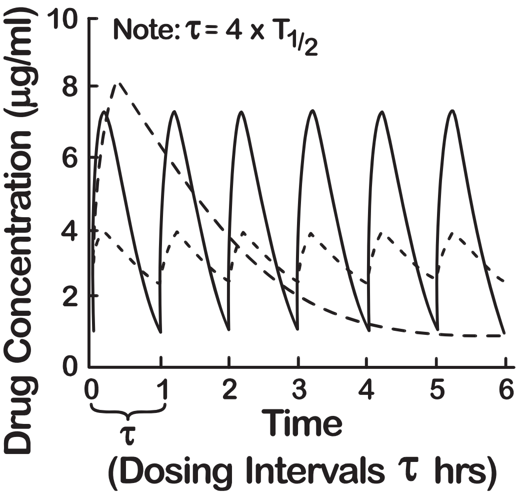

Figure 3.22 Comparison of constant dose (–––) and constant interval (----) regimens in renal failure (Cl_{cr} = one-sixth usual) with a normal dosage regimen (——) in a healthy patient. τ is the dosage interval.

The second method, *constant-dose, interval-extension method* (IE), extends the dosage interval by the inverse of the dose fraction, a value referred to as the *dose-interval multiplier:*

$$\begin{aligned} \tau_{renal\ failure} &= \tau_{normal}(1/K_f) \\ D_{renal\ failure} &= D_{normal} \end{aligned} \tag{3.81}$$

This type of dose adjustment strategy may also be implemented through the use of a nomogram where the dosage interval multiplier for this IE regimen is simply read off a plot of creatinine clearance. A comparison of these two methods is presented in Figure 3.22.

The therapeutic goal is to maintain a constant product of (T1/2 × D/τ) in healthy animals and those with renal failure. When this product is constant, the average steady-state plasma concentration of drug will remain unchanged. This is the approach followed in the DR and IE methods. A constant steady-state plasma concentration is achieved by the use of the dose fraction to compensate for decreased T1/2 in the following manner:

$$(T1/2\,D/\tau)_{normal} = K_f(T1/2\,D/\tau)_{renal\ failure} \tag{3.82}$$

When repeated doses of a drug are administered, accumulation occurs until steady-state plasma concentrations are achieved, a process taking five half-lives. The prolonged T1/2 present in patients with renal insufficiency would cause excessive delay in attaining steady-state concentration. Therefore, an appropriate loading dose should always be administered so that a therapeutic concentration of the drug is immediately attained. If the constant interval method is employed, this can be accomplished by giving the usual dose initially, followed by the calculated reduced dose. If the constant dose method is used, the initial two doses should be given according to the usual interval. The selection of which method to use is very drug dependent, both in terms of efficacy and potential toxicity.

The above equations hold for drugs that are excreted solely by the kidney, since the dose fraction adjusts dosages as if only renal elimination occurred. For drugs undergoing biotransformation, an estimate of the percent of nonrenal clearance is necessary. More detailed texts should be consulted for dealing with these cases. If hepatic disease is present, the same strategy holds if one has a marker for hepatic dysfunction. The problem is that there is no “creatinine” equivalent to assess the degree of functional hepatic impairment. One also must consider the hepatic extraction ratio and subsequent renal clearance of metabolites. Again, a specialty text in this area should be consulted.

Interspecies Extrapolations

The ultimate aim of any interspecies extrapolation would be to predict drug activity or toxicity in a new species not previously studied. There are two sources of error inherent to such an extrapolation. The first is that a drug’s pharmacokinetic profile (especially excretion, metabolism, and distribution) does not extrapolate across species without adjusting for some individual species characteristics. The second, which will always be problematic, is that the pharmacodynamic response of a drug may be very different between species and not at all related to pharmacokinetics. This latter concern may not be important for antimicrobial drugs since the pathogenic organisms being treated should have susceptibilities that are pathogen dependent and host independent. However, for drugs that interact with physiological functions that have species-specific receptor types and distributions, an estimate of pharmacokinetic parameters may not be sufficient to predict pharmacodynamic response.

There is a wealth of empirical observations that suggest that physiological functions such as O_2 consumption, renal glomerular filtration, cardiac output, etc., are not linearly correlated to the mass of an individual animal, both within and between species. That is, if one expresses any physiological function on a per kg body weight basis (e.g., GFR/kg), an *isometric* relationship would suggest that the parameter is constant. However, in the case of these physiological functions, such a relationship does not hold since the parameter on a mg/kg basis still is species dependent and not constant. A knowledge of body weight does not allow one to determine the value of the parameter across species with different body weights. However, if these parameters are expressed on a per unit surface area basis, many parameters such as GFR will be equivalent across species. More refined analyses suggest that the optimal scaling factor would be to a species’ Basal Metabolic Rate (BMR). Empirical observations suggest that BMR is a function of (body weight in Kg) raised to the 0.75 power [$GFR = \int -(BW_{kg})^{0.75}$]; when expressed on body surface area, the exponent is 0.67. An exponent of 0.75 is also theoretically predicted if metabolic functions are based on a model where substances in the body are transported through space-occupying fractal networks of branching tubes (e.g., the vascular system) that minimize energy dissipation and share the same size at the smallest level of structure (e.g., capillaries). Whatever the mechanism, these approaches are well suited for extrapolating drug disposition across species.

Equations where a parameter is related to a mathematical function (in this case, a power function) of a metric such as body weight is termed an *allometric* relationship. The extensive literature surrounding this question of how one “collapses” physiological parameters between species has created a field of study called *allometry*. Since most drug pharmacokinetic parameters are dependent upon some physiological function, they may also be scaled across species using these strategies. The method for doing this is to correlate the parameter of concern (e.g., GFR, Cl_B, T1/2 = most common) with body weight (BW) using the following allometric equation:

$$Y = a\,(BW)^b \tag{3.83}$$

where Y is the parameter of concern, a is the allometric coefficient and b the allometric exponent. The data is obtained using simple linear regression on $\log_{10}$ Y versus $\log_{10}$ BW, as depicted in Figure 3.23. The slope is the allometric exponent b and the intercept a. There is uniform agreement that for most physiological processes, the allometric exponent b ranges from 0.67 to 1.0. Note that if the parameter being modeled is an inverse function of a physiological process (e.g., T1/2), the exponent will be

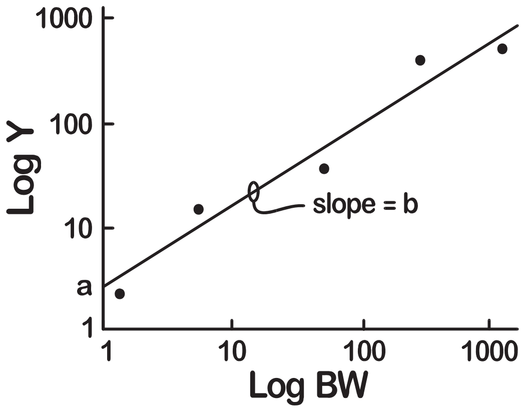

Figure 3.23 Basic log–log allometric plot of a biological parameter (Y) versus body weight (BW) with slope b and intercept a.

1 − b for that process. The coefficient a is actually the value of Y for a 1.0 kg BW animal (b = 0).

Numerous texts and research manuscripts deal with this topic in greater depth. There is an issue in human medicine where data is often extrapolated from small laboratory animals and dogs to larger humans, thus extrapolation error may be large. However, in veterinary medicine, one often interpolates because data are available in species of very different body masses. We recently published a large analysis of 85 drugs across multiple species that confirmed many of the factors just discussed (Huang et al., 2015). The important clinical take-home message to the veterinarian is that for equivalent effects, the dose may be greater in a smaller animal on a body weight basis. In cancer chemotherapeutics, doses are often expressed on the basis of body surface area, an adjustment that essentially compensates for the allometric exponent.

Conclusion

The purpose of this chapter was to introduce some core concepts of pharmacokinetics that determine the basis of dosage regimen construction in medicine. In most cases, a veterinarian will use the dosage recommended on the drug label. These concepts define where that dose originates, and importantly, how the clinician should consider adjusting it based on disease, therapeutic nonresponsiveness, or toxicity. The next chapter will present concepts linking a plasma concentration time profile to biological effect.

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4

Mechanism of Drug Action and Pharmacokinetics/Pharmacodynamics Integration in Dosage Regimen Optimization for Veterinary Medicine

Pierre-Louis Toutain

Introduction

Pharmacokinetics (PK) studies the fate of drugs in the animal whereas pharmacodynamics (PD) studies the action of drugs from its interaction with receptors, to the effect on animal populations. Pharmacokinetic/pharmacodynamic (PK/PD) integration consists of describing and explaining the time course of the drug effect (PD) via the time course of its concentration in the plasma (PK). For a systemic drug, a tenet in pharmacology is that the plasma concentration controls the concentration at the site of action (the so-called *biophase* where receptors, pathogens, parasites, etc. are located) and that a proportional relationship exists between the plasma and biophase concentrations at equilibrium. This is why plasma concentration, which is easy to measure, can be used as an explicative variable of the in vivo drug effect when integrating PK and PD data in a PK/PD modeling approach (Figure 4.1). Plasma concentration, unlike the biophase concentration, is also easy to control with an appropriate dosage regimen and one of the main goals of PK/PD analysis is to precisely determine the dosage regimen (dose, dosage interval). The ultimate goal in controlling the plasma drug concentration (also termed drug exposure or internal dose) is to achieve some expected endpoint in terms of drug efficacy and safety.

The first part of this chapter addresses the question of drug pharmacodynamics with special emphasis on the relationship between drug concentration and intensity of action at the receptor level. The second part of this chapter is devoted to the in vivo situation and to the whole drug response. The classical dose-titration methods for dose determination and their limits are described with special emphasis on the alternative PK/PD approaches. The use of PK/PD modeling in studies of veterinary drugs has been reviewed (Riviere and Toutain, 2011; Toutain, 2002; Toutain and Lees, 2004) for antibiotics (Lees et al., 2006; McKellar et al., 2004; Toutain et al., 2002), angiotensin converting enzyme (ACE) inhibitors (Toutain and Lefebvre, 2004) and nonsteroidal antiinflammatory drugs (NSAIDs) (Lees et al., 2004a; Lees et al., 2004b). A glossary of pharmacodynamic terms and symbols is given in (Lees et al., 2004a; Neubig et al., 2003).

Types of Drug Targets

Most drugs act via an interaction with certain proteins either of the host or of the pathogen (Figure 4.2). Detailed discussions of drug mechanism of action are discussed in the specific chapters of this text. Exceptions are drugs in which the activity is based on physical properties, such as osmotic diuretics (e.g., mannitol) and antacids. Protamine that can be injected as an antidote of heparin acts as a physical antagonist by binding to it. Tiludronic acid is a biphosphonate used to prevent or to treat a variety of bone conditions. It binds to hydroxyapatite crystals and inhibits hydroxyapatite breakdown, suppressing bone resorption (Drake et al., 2008). General anesthetics were previously thought to produce their effect by simply dissolving in the lipid bilayer of the nerve membrane. It is currently acknowledged that all anesthetics act by either enhancing inhibitory signals or by blocking excitatory signals (for a review see Garcia et al., 2010). For intravenous anesthetics such as propofol and inhaled anesthetic as isoflurane, the target has been identified as the $\mathrm{GABA_A}$ receptor ($\mathrm{GABA_R}$), the most abundant fast inhibitory neurotransmitter receptor in the central nervous system. Ketamine and nitrous oxide inhibit ionotropic glutamate receptors, with the strongest effects being seen on the NMDA receptor subtype.

Four types of protein are targeted by drugs: *enzymes, carriers, ion channels,* and *receptors* (Figure 4.2). The term receptor should be reserved for regulatory proteins that play a role in intercellular communication. Thus, enzymes, ion channels, and carriers are not usually classified as receptors.

Enzymes such as cyclooxygenases are the target site for NSAIDs and their inhibition leads to the suppression of

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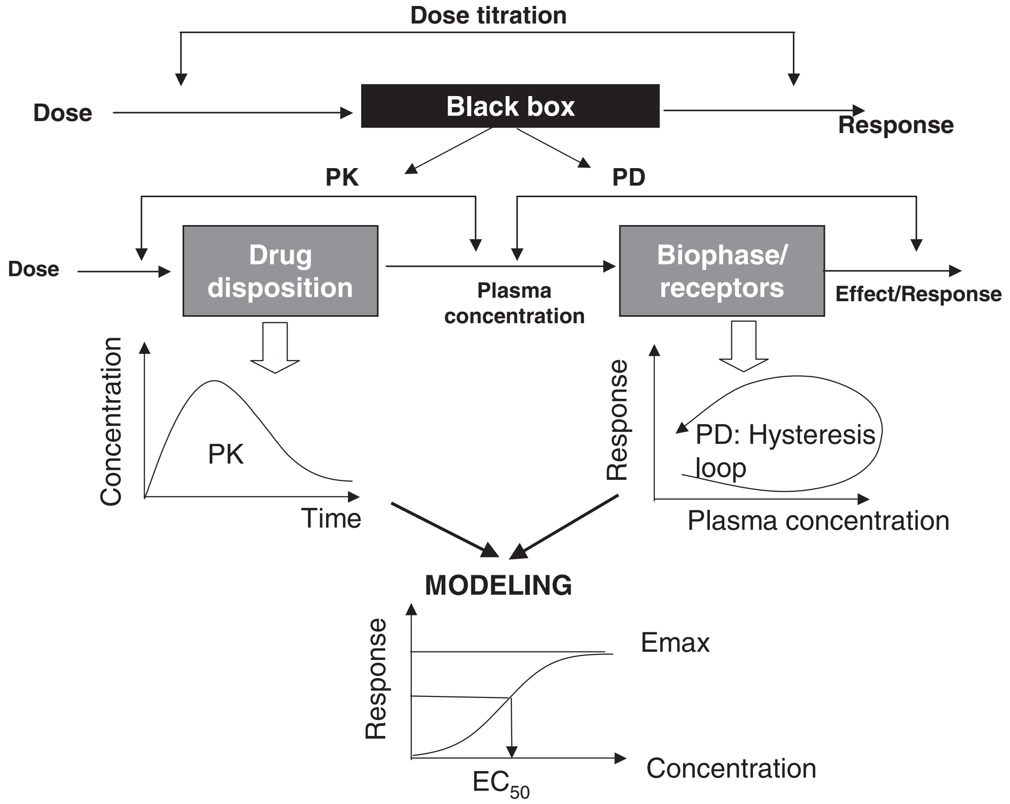


Figure 4.1 Dose–effect relationship versus PK/PD modeling. Both approaches aim at documenting the same relationship between dose and drug response. A dose–effect relationship is a black-box approach in which the dose is the explicative variable of drug response. In a PK/PD approach, the black box is opened, thereby enabling the two primary processes that separate dose from response to be recognized. In the first step (PK), the dose is transformed into a plasma concentration profile. In the second step (PD), the plasma concentration profile becomes the variable, which explains the drug response. The difficulty with the PK/PD approach is that the development of effect and plasma concentrations over time is usually not in phase. This means that a hysteresis loop is observed when the response is plotted against plasma concentrations and data modeling is required to estimate the PD parameters (E_{max}, ED_{50}, and slope).

proinflammatory prostaglandins. Acetylcholine esterase, an enzyme that metabolizes acetylcholine at the receptor site, is a target site for cholinesterase inhibitors (neostigmine, physostigmine, etc.). Cholinesterase inhibitors act indirectly by preventing the enzyme from hydrolyzing acetylcholine. Other examples of enzymes serving as drug targets are dihydrofolate reductase for trimethoprim (an antibacterial) and angiotensin converting enzyme (ACE) for ACE inhibitors such as benazepril and enalapril. Antibiotics may act by inhibiting enzymes involved in cell wall biosynthesis, nucleic acid metabolism and repair, or protein synthesis, which is why antibiotics are generally more efficacious on multiplying bacteria.

Carriers (also termed membrane transport proteins) are target sites for many drugs. The $Na^+/K^+/2Cl^-$ symport in the nephron is the site of action of furosemide and other loop diuretics such as torasemide. Furosemide acts at the luminal surface of the thick ascending limb of the loop of Henle to prevent sodium chloride reabsorption. For all diuretics other than spironolactone, the biophase is urine, not plasma, and they need to gain access to the lumen of the nephron to develop their diuretic action. This explains why in renal failure, the dose often needs to be substantially increased, not decreased as for other drugs. ATP-powered ion pumps such as the sodium pump (Na^+/K^+ ATPase) are the target sites for cardioactive digitalis and the Na^+/H^+ pump in the gastric parietal cell is the target site for proton pump inhibitors such as omeprazole.

Some drugs, such as local anesthetics, produce their effects in the nervous system and in the heart (Grace and Camm, 2000; Sills, 2011) by directly interacting with *ion channels*. They inhibit *voltage-gated* Na^+ *channels* in sensory neurons by binding to specific sites within the Na^+ channel and produce a direct effect by incapacitating the protein molecule. Their affinity varies with the gating state of the channel, with a high affinity when the channels are opened and inactivated during action potentials at high frequency, as occurs during pain or a cardiac arrhythmia. Most antiepileptic drugs act on voltage–gated Na^+ channels and voltage-dependent

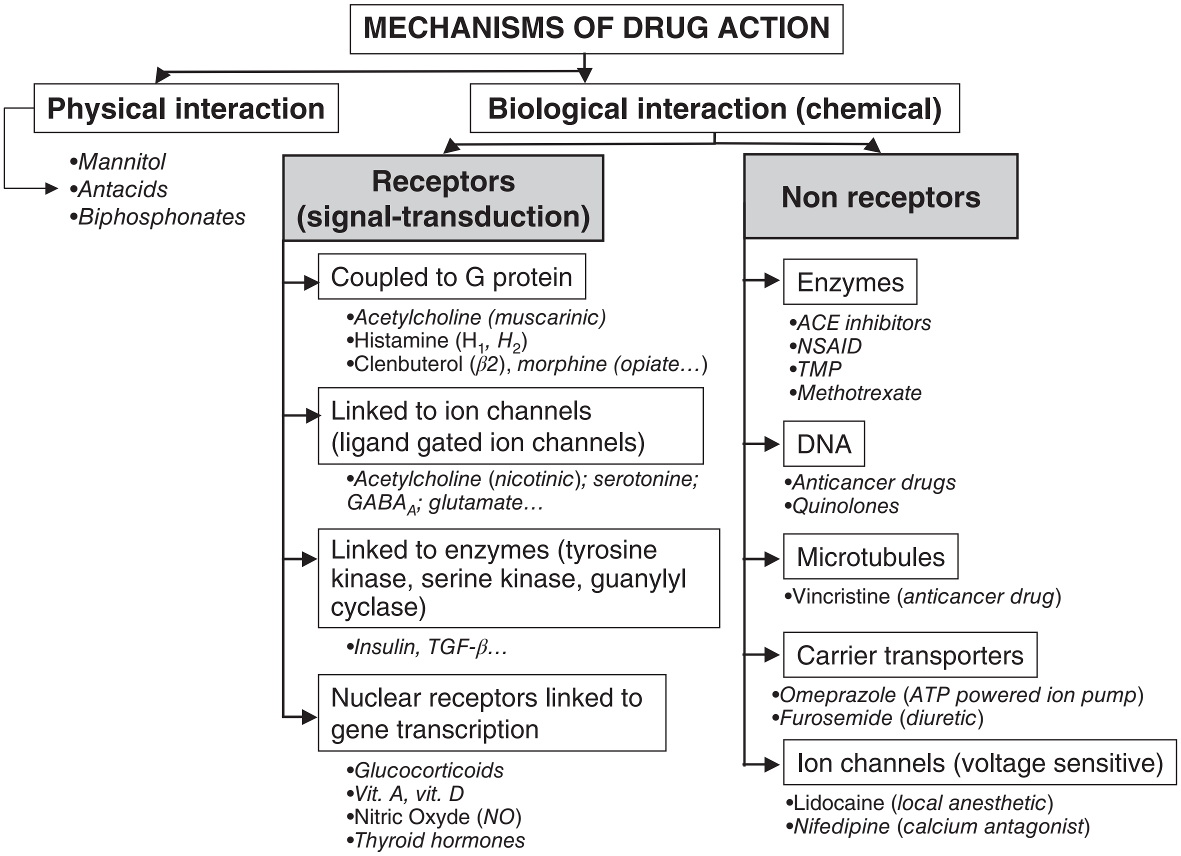

MECHANISMS OF DRUG ACTION
Physical interaction
•Mannitol
•Antacids
•Biphosphonates
Biological interaction (chemical)
Receptors (signal-transduction)
Non receptors
Coupled to G protein
•Acetylcholine (muscarinic)
•Histamine (H_1, H_2)
•Clenbuterol (β2), morphine (opiate…)
Linked to ion channels (ligand gated ion channels)
•Acetylcholine (nicotinic); serotonine; $GABA_A$; glutamate…
Linked to enzymes (tyrosine kinase, serine kinase, guanylyl cyclase)
•Insulin, TGF-β…
Nuclear receptors linked to gene transcription
•Glucocorticoids
•Vit. A, vit. D
•Nitric Oxyde (NO)
•Thyroid hormones
Enzymes
•ACE inhibitors
•NSAID
•TMP
•Methotrexate
DNA
•Anticancer drugs
•Quinolones
Microtubules
•Vincristine (anticancer drug)
Carrier transporters
•Omeprazole (ATP powered ion pump)
•Furosemide (diuretic)
Ion channels (voltage sensitive)
•Lidocaine (local anesthetic)
•Nifedipine (calcium antagonist)

Figure 4.2 Mechanisms of drug action. For most drugs, the drug action is mediated by some biological interaction with a macromolecule of the cell, often a protein. Different proteins are involved as drug targets and the term receptor is only used when the interaction triggers a cascade of events for signal transmission.

gated calcium channels are the main target of most calcium-channel blockers, such as the antiarrhythmic drugs verapamil and amlodipine. This mechanism of drug action on ion channels should not be confused with that of *ligand-gated ion channels,* which function as ionotropic receptors (see Section Macromolecular Nature of Drug Receptors).

Other nonreceptor/nonprotein targets as sites of action are nucleic acids for drugs such as actinomycin D, an antineoplastic antibiotic. DNA is also the target for a number of antibiotics (quinolones) as well as mutagenic and carcinogenic agents.

Drug Receptor and Ligand as Agonist or Antagonist

A receptor is a molecule or a polymeric structure on the surface of or inside a cell that specifically recognizes and binds an endogenous compound. Binding sites are three-dimensional structures, forming pockets or grooves on the surface of protein that allow specific interactions with compounds known as *ligands*, which are molecules of complementary shape to the protein binding site (lock and key analogy). Receptors possess an effector system (also termed signal-transduction pathways). In this they differ from *acceptors* which are molecules without signal-transduction pathways (e.g., serum albumin), characterized by a binding process that is not followed by a physiological response.

Endogenous neurotransmitters, such as hormones, act as molecular messengers and are endogenous ligands. Drugs may be viewed as exogenous ligands. After attachment to a receptor site, a drug may produce a cascade of biochemical events that result in drug action. A drug is said to be an *agonist* when it produces a measurable physiological or pharmacological response characteristic of the receptor (contraction, relaxation, secretion, enzyme activation, etc.). If a drug binds to the same site as the endogenous ligand, the drug is said to be a *primary agonist,* in comparison to an *allosteric agonist* which binds to a different region of the receptor. Most anesthetic drugs allosterically modulate $GABA_R$ and disrupt corresponding physiological circuits. A *full agonist* produces a maximal effect under a given set of conditions whereas a *partial agonist* produces only a submaximal effect regardless

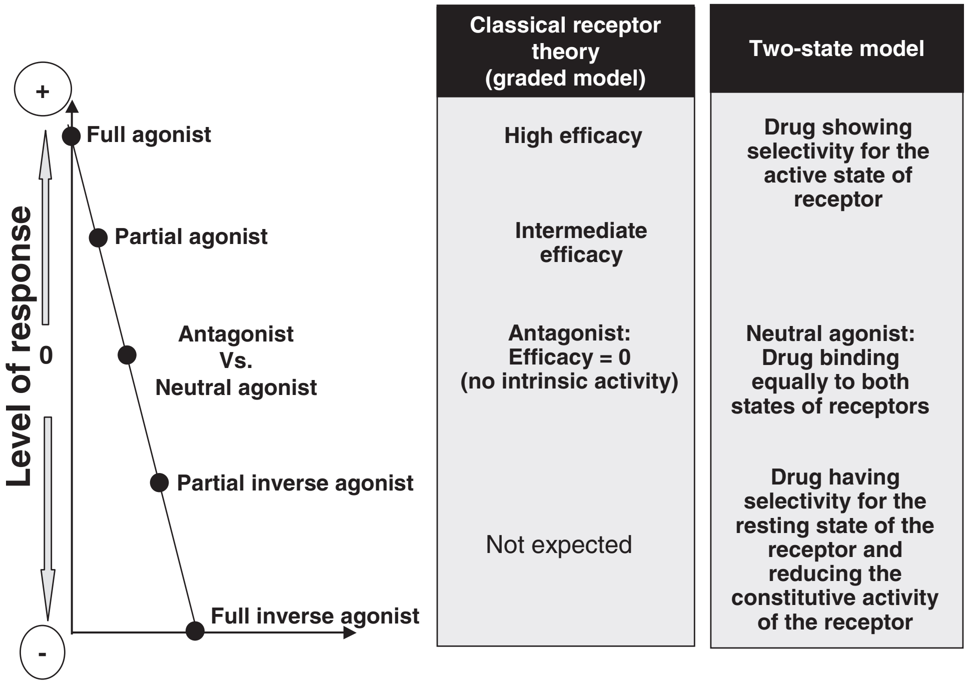

Classical receptor theory (graded model)
Two-state model
+
Level of response
0
-
Full agonist
Partial agonist
Antagonist Vs. Neutral agonist
Partial inverse agonist
Full inverse agonist
High efficacy
Intermediate efficacy
Antagonist: Efficacy = 0 (no intrinsic activity)
Not expected
Drug showing selectivity for the active state of receptor
Neutral agonist: Drug binding equally to both states of receptors
Drug having selectivity for the resting state of the receptor and reducing the constitutive activity of the receptor

Figure 4.3 Schematic representation of the spectrum of drug effect from full agonist (maximum positive effect) to full inverse agonist (maximum negative effect). Such a spectrum is only seen if a constitutive receptor activity exists which can be inhibited by an inverse agonist (see Figure 4.9 for explanation). According to the "receptor theory", drugs may be agonists or antagonists. A full agonist produces full receptor activation leading to a maximal response; a partial agonist produces a submaximal response and possible blockade of a full agonist activation. An antagonist does not produce any physiological response but is able to block the response of an endogenous ligand or exogenous agonist. In the framework of the two-state model, any drug is viewed as an agonist, and an antagonist is a neutral agonist producing no response. According to this model "efficacy" is genuinely explained by the relative affinity of the drug for one of the states of the receptor (activated or resting state) and inverse agonists are drugs showing selectivity for the resting state of the receptor. An inverse agonist acts as an antagonist but has the supplementary property over a classical antagonist of reducing receptor-mediated constitutive activity.

of the amount of drug applied. For opioid receptors, morphine and fentanyl are full agonists, able to initiate strong analgesia, while buprenorphine is a partial agonist (Lees et al., 2004b). Even if buprenorphine is unable to achieve the same level of analgesia provided by a maximally effective dose of full agonists, it may be preferred for postsurgical analgesia because its effect is of long duration and adverse effects are minimal.

In contrast to an agonist, some drugs may be unable to trigger any action on their own, after attachment to a receptor site, but are able to block the action of other agonists. These "silent drugs" are termed *antagonists*; most drugs used in therapeutics are receptor antagonists and prevent the action of natural agonists (neurotransmitters, hormones, etc.). Some drugs may be both agonist and antagonist, for example butorphanol, a central-acting opioid analgesic, is mainly an antagonist at the *mu* receptor but is an agonist at the *kappa* receptor. Two forms of antagonism can be distinguished: *competitive* and *noncompetitive antagonism*. In competitive antagonism, antagonists act on the same receptor as the agonist; this is said to be reversible when it can be surmounted by increasing the concentration of the agonist. Examples of therapeutic agents acting by competitive antagonism are atropine (an antimuscarinic agent) and propranolol (a beta blocker). In irreversible (competitive) antagonism, a net displacement of the antagonist from its binding site cannot be achieved by increasing the agonist concentration and, operationally, it resembles noncompetitive antagonism. This occurs when the antagonist is bound covalently and irreversibly to its receptor binding site. Although there are few drugs of this type, irreversible antagonists are used as experimental probes for investigating receptor function. *Noncompetitive antagonism* refers to the situation where a drug blocks the cascade of events, normally leading to an agonist response, at some downstream point. This occurs with Ca^{2+} channel blockers, such as nifedipine, which prevent the influx of calcium ions through the cell membrane and nonspecifically block any agonist action requiring calcium mobilization, as in smooth muscle contraction. The concept of *physiological antagonism* refers to the interaction of two drugs whose opposing actions on a physiological system tend to cancel each other out. For example, histamine acts on receptors of the parietal cells of the gastric mucosa to stimulate acid secretion, while omeprazole blocks this effect by inhibiting the proton pump.

Antagonists were viewed solely as “silent ligands” until the discovery of the so-called inverse agonists. An inverse agonist is a drug that acts at the same receptor as that of an agonist, yet produces an opposite effect (see Section Drug Receptor Theories: from the Occupancy Theories to the Two-State Model for the mechanism of inverse agonist action). Figure 4.3 summarizes the spectrum of activities that ligands can display.

Drug Affinity, Efficacy and Potency

The concentration–effect relationship is determined by two features of the drug–receptor interaction, namely drug *affinity* and drug *efficacy*. The affinity of a drug is its ability to bind to a receptor. Affinity is determined by the chemical structure of the drug and minimal modification of the drug structure may result in a major change in affinity. This is exploited to discover new drugs. Affinity determines the concentration of drug required to form a significant number of drug–receptor complexes that in turn are responsible for drug action. The numerical representation of affinity for both an agonist and an antagonist is the *constant of affinity,* denoted by Ka (dimension M^{-1}, i.e., liter per mole). A Ka of $10^7 M^{-1}$ means that one mole of the ligand must be diluted in 10^7 liters of solvent to obtain a concentration of the free ligand able to saturate half the maximal binding capacity of the system. The reciprocal of Ka is the equilibrium *dissociation constant* of the ligand–receptor complex, denoted by Kd (dimension M i.e., mole per liter). A Kd of 10^{-7}M means that a free ligand concentration of 10^{-7} mole per liter is required to saturate half the maximal binding capacity of the system. The lower the Kd value of a drug, the higher the affinity for its receptor.

Radioactive receptor ligands (radioligands) or fluorescent probes are used to accurately determine receptor affinity. The relationship between bound and free ligand may be described by a hyperbolic equation (Figure 4.4) corresponding to the Michaelis Menten equation:

$$Bound = \frac{B\max \times Free}{Kd + Free} \tag{4.1}$$

Where B_{max} (a parameter) represents the maximal binding capacity (the total number of receptors), *Free* is the molar concentration of the free ligand, *Bound* is the bound ligand concentration and *Kd* (a parameter) is the equilibrium constant of dissociation.

From this equation, the receptor occupancy (i.e., the fraction of receptor occupied) can be described by:

$$fraction\ occupied = \frac{Bound}{B\max} = \frac{Free}{Free + Kd} \tag{4.2}$$

This is known as the Hill–Langmuir equation.

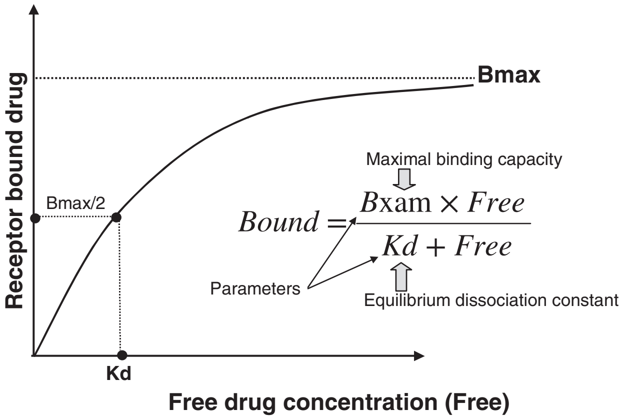


Figure 4.4 Relationship between free drug concentration (*Free*) (independent variable), that is the driving concentration, and receptor-bound drug (dependent variable). Affinity of a drug for its receptor is expressed by Kd (a low Kd means a high affinity). B_{max} indicates the maximal binding capacity of the receptor.

The generation of a response for the drug–receptor complex is governed by a property named efficacy. *Efficacy* is the drug's ability, once bound, to initiate changes that lead to the production of responses. It is a property of the ligand/receptor pair. This term is used to characterize the level of maximal response (E_{max}) induced by an agonist. In contrast, a pure antagonist has no *intrinsic efficacy* because it does not initiate a change in cell functions. This concept of efficacy is not to be confused with the drug's *clinical efficacy* whereby an antagonist may be fully efficacious. This is because blocking the binding of an endogenous agonist to the receptor by an antagonist may be clinically useful. *Potency* corresponds to the concentration of drug required to achieve a given effect. It is expressed by the EC_{50} (or the IC_{50} if the effect is an inhibition), that is the concentration of an agonist which produces 50% of the maximum possible response for that agonist (Figure 4.5). Potencies of drugs vary inversely with the numerical value of their EC_{50} and the most potent drug is the one with the lowest EC_{50}.

The concept of drug efficacy and potency is also used in a clinical context. Drug efficacy is the property of most interest to clinicians who are looking for the most efficacious drug. For example, it was shown using an inflammatory model in horses that flunixin was more efficacious than phenylbutazone in induced lameness (Toutain et al., 1994). For some drugs such as loop diuretics, there is actually a maximum possible effect that can be obtained regardless of how large a dose is administered and this is termed the *high ceiling effect.* When different drugs within a series are compared, the most potent drug is not necessarily the most clinically efficacious (Figure 4.6). For example, butorphanol is more potent but less efficacious than morphine for analgesia. Another example is glucocorticoids. All glucocorticoids

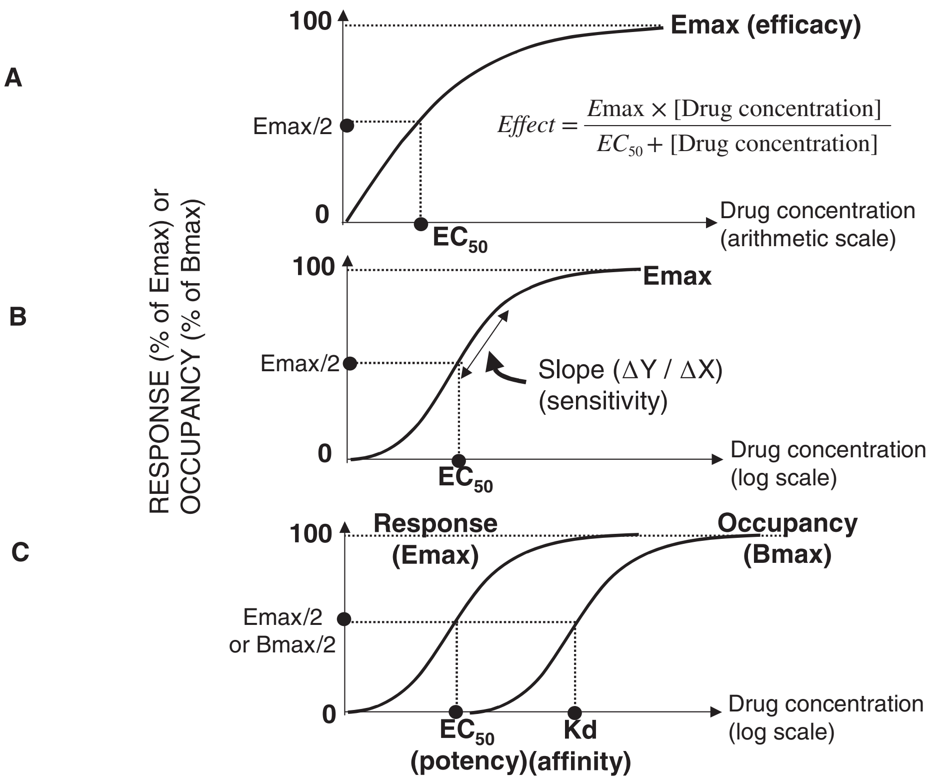


Figure 4.5 The dose–response relationship and definition of the three main pharmacodynamic parameters.
(A) When the drug response (arithmetic Y axis) is plotted against the tested dose (arithmetic X axis), a hyperbolic relationship is often observed with a maximal effect denoted E_{max}. EC_{50} is the concentration that produces an effect equal to half E_{max}.
(B) When the same data are represented using an X log scale, the relationship becomes sigmoidal with a more or less steep slope. The advantage is to compress the dose scale and to visualize the slope more easily. A sigmoidal curve allows the definition of three drug parameters, namely E_{max}, ED_{50}, and the slope (ΔY/ΔX). E_{max} describes the drug efficacy and EC_{50} describes the drug potency. The slope (measured by the *n* of the Hill equation; see Equation 4.4 in the text) indicates the sensitivity of the dose–effect curve. The slope of the curve is involved in the drug selectivity (see Figure 4.11).
(C) This plot shows that the concentration effect curve is situated to the left of the free concentration (occupancy)-bound concentration curve with EC_{50} generally $\ll$ Kd.

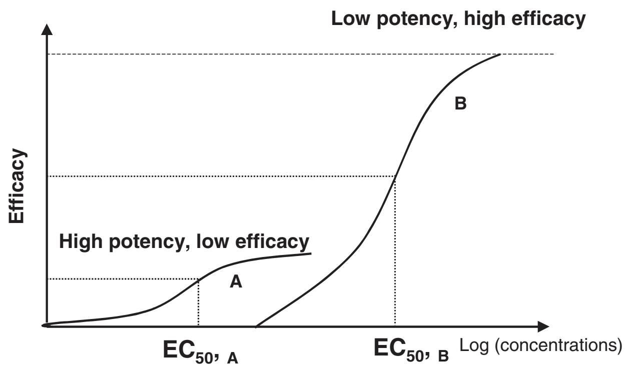


Figure 4.6 Drug potency versus drug efficacy. These two terms are often confused. Potency is expressed in terms of concentration (X axis) and EC_{50} is the parameter measuring drug potency. Efficacy (Y axis) expresses the level of response with a maximal possible effect (E_{max}). Efficacy is the parameter of interest for a clinician but potency may be a limiting factor if the drug has to be administered in a small volume. Here drug A has a higher potency than drug B but has a lower efficacy.

are able to totally suppress the endogenous cortisol secretion (adrenal suppressive effect), as well as to ensure a full eosinopenia, thymus involution and in regard to these suppressive endpoints, they all have the same pharmacological efficacy. However, their potencies are very different, with dexamethasone, betamethasone, and triamcinolone acetonide being 20 to 30-fold more potent than cortisol, whereas prednisolone and methylprednisolone are only four to fivefold more potent than cortisol. A low potency is only a disadvantage when the effective dose is too large to be convenient; for example for a spot-on, eye drop or intraarticular administration, the volume to be administered must be small and only relatively potent drugs can be administered in this way.

Drug Specificity and Selectivity

The drug receptor interaction is responsible for the *specificity* and *selectivity* of drug action. When the drug acts only on a single target (enzyme, receptor, etc.), it is said to be specific. *Specificity* is linked to the nature of the drug–receptor interaction and more precisely to the macromolecular structure of the receptors (or enzymes). As receptors are generally proteins, the diversity in three-dimensional shape required for ligand specificity is provided by the polypeptide structure. The recognition of specific ligands by receptors is based on the complementarity of the three-dimensional structure of the ligand and a binding pocket on the macromolecular target. The shapes and actions of receptors are currently being investigated by X-ray crystallography and computer modeling.

Specificity is rare. Most drugs can display activity towards a variety of receptors and are more often selective than specific. For example histamine antagonists produce several effects, such as sedation and prevention of vomiting, which do not depend on histamine antagonism. *Selectivity* is related to the concentration range. The drug may be specific at a low concentration if it activates only one type of target, whereas several targets may be involved simultaneously (activated, inhibited, etc.) if the drug concentration is increased. This is the case with NSAIDs and inhibition of the different subtypes of cyclooxygenases (COX-1 vs. COX-2 isoenzymes) (Figure 4.7). Cortisol possesses both glucocorticoid and mineralocorticoid properties and cortisol at pharmacological dose causes unwanted side effects, such as fluid–electrolyte imbalance, which is the reason why it cannot be used as an antiinflammatory drug. This is due to the close structural relationship of the nuclear glucoreceptor (GR) and the mineralocorticoid receptor (MR). The in vivo natural ligand of the MR is aldosterone. A biological paradox is that the affinity for the MR is similar for aldosterone and cortisol, while cortisol concentration in epithelial tissues is much higher (100–1000 fold) than aldosterone concentration, raising the issue of a possible unwanted binding of cortisol to the MR. Actually, the in vivo selectivity of aldosterone for the MR exists despite its much lower concentration and is of enzymatic origin; it is determined by the intracellular metabolism of cortisol into cortisone, an inactive metabolite with a low affinity for the MR. This inactivation of cortisol is carried out by the unidirectional 11β-hydroxysteroid dehydrogenase (11βHSD) of type 2, an enzyme expressed in the epithelial aldosterone target cell. 11βHSD can be inhibited by various substances, such as furosemide and licorice. For synthetic glucocorticoids, it was desirable to separate the gluco- from the mineralocorticoid effect and selectivity for the GR (associated to the antiinflammatory effect) was obtained by structural modification of the cortisol; first an increase of the selectivity was obtained by the introduction of Δ1-dehydro configuration (as for prednisolone) and then by adding a hydrophobic residue in position 16 as a 16-α-methyl group for dexamethasone or a 16-β-methyl group for betamethasone and beclomethasone(Farman and Bocchi, 2000).

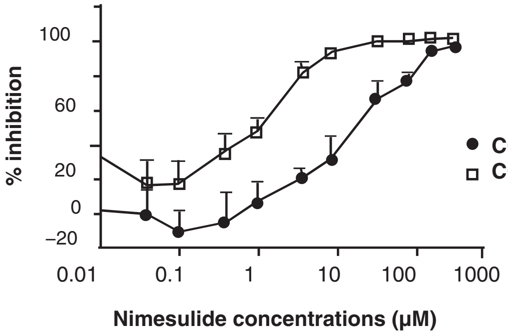


Figure 4.7 Drug selectivity. For a non steroidal antiinflammatory drug (NSAID) such as nimesulide, the antiinflammatory properties are linked to the inhibition of cyclooxygenase (COX). There are two isoforms of COX, namely COX-1 and COX-2. COX-2 is an inducible enzyme formed at the site of inflammation and producing proinflammatory prostaglandins such as PGE$_2$, whereas COX-1 is a constitutive enzyme that performs a range of housekeeping functions. The selective inhibition of COX-2 is considered the primary action to be achieved for a NSAID and the nonselective inhibition of COX-1 is considered detrimental, especially for the digestive tract (ulceration). This COX-2/COX-1 selectivity can be explored ex vivo using a whole blood assay (Toutain et al., 2001b). When this assay was conducted using canine blood, the production of TxB$_2$ by platelets was used to measure COX-1 inhibition and the production of PGE$_2$ from LPS-stimulated leukocytes was used to measure COX-2 inhibition. The IC$_{50}$ values were 1.6 ± 0.4 µM for COX-2 and 20.3 ± 2.8 µM for COX-1, indicating that nimesulide is about 12 times more potent for COX-2 than for COX-1 isoenzymes. Nevertheless, nimesulide cannot be considered as a selective COX-2 inhibitor but only as a preferential COX-2 inhibitor. COX-2 would need to be almost totally inhibited to obtain a full antiinflammatory effect and this is impossible to achieve without any COX-1 inhibition. *Source:* Toutain 2001b. Reproduced with permission of John Wiley & Sons, Inc.

Lack of selectivity in vivo may lead to an overall complex and unpredictable pharmacological response and even undesirable side effects. This is the case, for example, for drugs administered as racemic mixtures of stereoisomers. Stereoisomers can exhibit not only very different potency and efficacy for a given effect, but also very different dispositions with possible interconversion between stereoisomer, rendering it difficult to predict the net effect of the drug and its time development. For antibiotics, antiparasitics, and similar drugs, the concept of selectivity is twofold: selectivity regarding the host versus selectivity regarding the target. Pathogen selectivity is generally expressed in terms of the spectrum of activity using an classification tool such as the staining properties (Gram positive vs. Gram negative) or a taxonomical classification (family, species, strains, etc.). Selectivity regarding the host is also an important characteristic to minimize the toxicity of antibiotics and insecticides. For example, penicillin inhibits key enzymes (transpeptidases, carboxypeptidases) involved in the peptidoglycan biosynthesis required for synthesis of the bacterial wall. These enzymes do not exist in mammals, ensuring an excellent selectivity and safety of this class of antibiotics in mammals.

Assessing the therapeutic usefulness of a new drug requires the determination of its full spectrum of biological activities towards a variety of relevant targets. The next task is then to explore this in vivo, and select an appropriate dosage regimen that maintains the plasma drug concentration within the range in which only the desired response is expressed (i.e., within the so-called *therapeutic window*).

Chemical Forces and Drug Binding

Several chemical intermolecular forces such as ionic bonds, hydrogen bonds, and Van der Waals forces may be involved in reversible binding of the drug to the receptor. In contrast, drug–receptor interactions involving covalent bond formation (very tight) are generally irreversible. Covalent binding to receptors is relatively rare. However, covalent binding is more frequent for drugs acting on enzymes. COX-1 inhibitors are generally reversible, although aspirin acts as a noncompetitive inhibitor of platelet COX-1 through a covalent binding mechanism. This is achieved by irreversible acetylation and explains the duration of the action of aspirin on blood coagulation. As platelets have no nucleus, the effect of aspirin is reversed by the production of new platelets. Omeprazole (a proton pump inhibitor) is another example of irreversible binding to an enzyme (H^+,K^+-ATPase). Drugs that bind covalently to DNA (alkylating agents) are extensively used as anticancer drugs.

Since many drugs contain acid or amine functional groups that are ionized at physiological pH, ionic bonds are formed by the attraction of opposite charges in the receptor site. Ionic bonds are the strongest noncovalent bonds. The attraction of opposite charges is brought about by polar–polar interactions as in hydrogen bonding. Although this electrostatic interaction is weaker than the ionic bond, an important feature of hydrogen bonding is the structural constraint. Thus the formation of hydrogen bonds between a drug and its receptor provides some information about the three-dimensional structure of the resulting complex. The same forces are responsible for the shape of the protein and for its binding properties, so shape influences binding and, in turn, binding can influence protein shape. The ability of protein to change shape is called allostery.

Macromolecular Nature of Drug Receptors

Many receptors are transmembrane proteins embedded in the lipid bilayer of cell membranes. They have two functions: ligand binding and message transduction. Transmembrane receptors include metabotropic and ionotropic receptors (Figure 4.8). The four superfamilies of receptor proteins include: (i) ligand-gated ion channels, which are membrane-bound receptors, directly linked to an ion channel; (ii) G-protein coupled receptors, which are membrane-bound receptors coupled to G-proteins; (iii)tyrosine kinase-linked receptors, which are membrane bound receptors containing an intrinsic enzymatic function (tyrosine kinase activity) in their intracellular domain; and (iv) transcription factor receptors that are intracellular receptors regulating gene transcription.

The activation of metabotropic receptors (tyrosine kinase receptors and G-protein-coupled receptors) leads to some changes in metabolic processes within the cell, whereas ionotropic receptors directly open or close an ion channel. When an ionotropic receptor is activated, it opens a channel that immediately allows ions such as Na^+, K^+, or Cl^- to flow. In contrast, when a metabotropic receptor is activated, a series of intracellular events is first triggered that may also subsequently result in ion channel opening.

G-protein-coupled receptors (GPCRs), also known as *seven transmembrane receptors (7TM receptors)*, transduce an extracellular signal (ligand binding) into an intracellular signal (G-protein activation). The signal is transferred via conformational alterations to a member of the family of G-proteins. G-proteins may act directly on an ionic channel or activate an enzymatic system (adenylyl cyclase, guadenylyl cyclase, phospholipase C, etc.) to release a range of second messengers (cAMP, cGMP, IP3, etc.), which ultimately permits certain ions to enter or leave the cell. Muscarinic acetylcholine receptor, adrenoceptors (α_1, α_2, β), dopamine, histamine, opioids, ACTH, and other receptors are examples of 7TM receptors.

The so-called *receptor tyrosine kinase* (RTK) family is also a class of metabotropic cell surface receptors, which exert their regulatory effect by phosphorylating different effector proteins. An RTK consists of an extracellular binding site and an intracellular portion with enzymatic activity (tyrosine kinase, serine kinase) and usually spans the cell membrane once only. Tyrosine kinase enzymes can transfer a phosphate group from ATP to a tyrosine residue in an intracellular protein to increase its phosphorylation. Protein phosphorylation is one of the underlying mechanisms regulating protein function. It can alter the biological properties or the interaction of proteins with other proteins or peptides. This family of receptors includes insulin, IGF-1, cytokines, and epidermal growth factor. The hormones and growth factors that act on this class of receptors are generally growth promoting and function to stimulate cell division.

Ionotropic receptors activate transmembrane ion channels. They contain a central pore, which functions as a ligand-gated ion channel. Nicotinic cholinergic receptor, $GABA_A$ receptor, and glutamate, aspartate, and

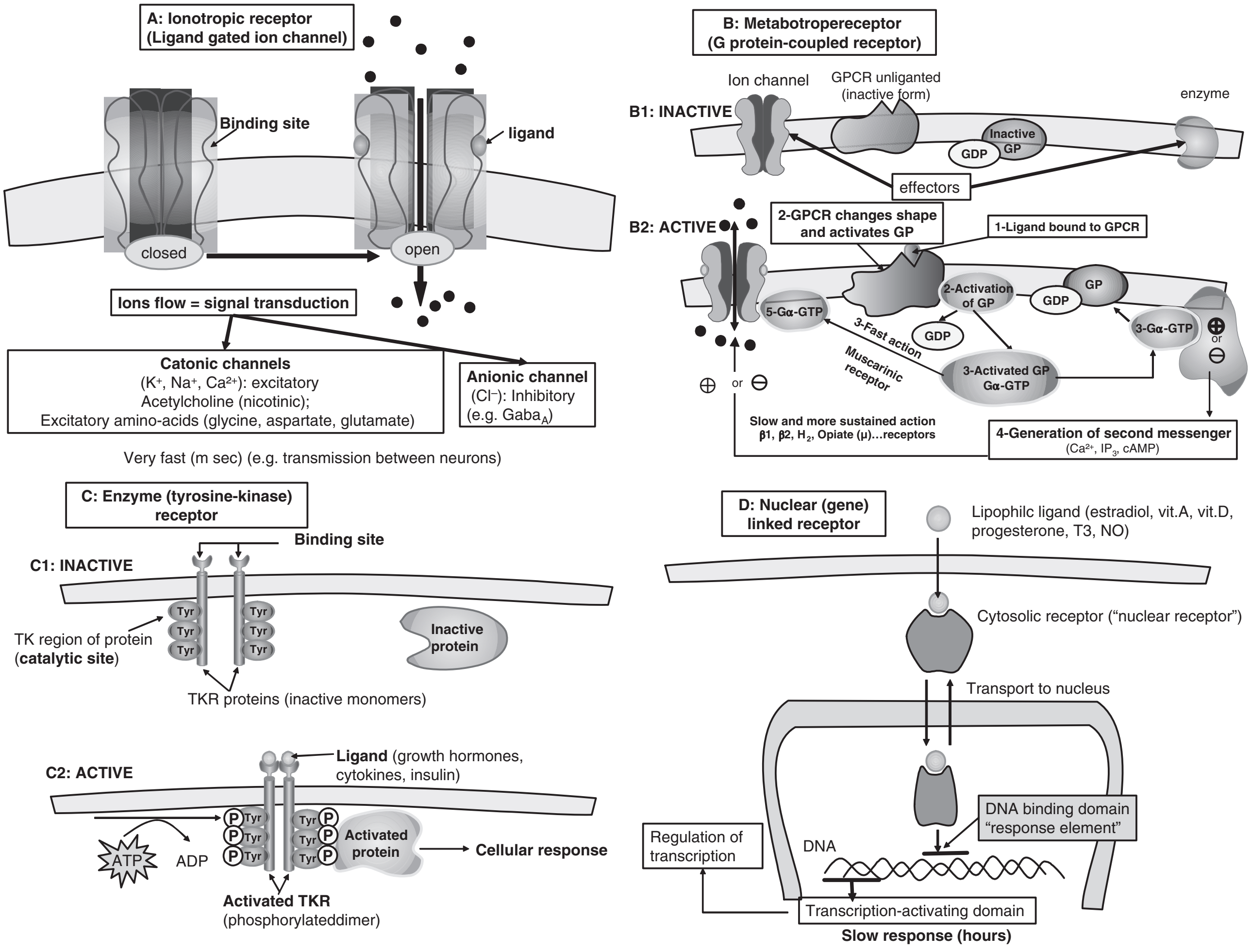

A: Ionotropic receptor
(Ligand gated ion channel)
Binding site
ligand
closed
open
Ions flow = signal transduction
Catonic channels
(K+, Na+, Ca2+): excitatory
Acetylcholine (nicotinic);
Excitatory amino-acids (glycine, aspartate, glutamate)
Anionic channel
(Cl−): Inhibitory
(e.g. GabaA)
Very fast (m sec) (e.g. transmission between neurons)
B: Metabotropereceptor
(G protein-coupled receptor)
Ion channel
GPCR unliganted
(inactive form)
enzyme
B1: INACTIVE
Inactive GP
GDP
effectors
B2: ACTIVE
2-GPCR changes shape and activates GP
1-Ligand bound to GPCR
2-Activation of GP
GDP
GP
5-Gα-GTP
3-Fast action
Muscarinic receptor
GDP
3-Activated GP Gα-GTP
3-Gα-GTP
⊕ or ⊖
⊕ or ⊖
Slow and more sustained action
β1, β2, H2, Opiate (μ)…receptors
4-Generation of second messenger
(Ca2+, IP3, cAMP)
C: Enzyme (tyrosine-kinase) receptor
Binding site
C1: INACTIVE
Tyr
TK region of protein
(catalytic site)
Inactive protein
TKR proteins (inactive monomers)
C2: ACTIVE
Ligand (growth hormones, cytokines, insulin)
P
Tyr
Activated protein
Cellular response
ATP
ADP
Activated TKR
(phosphorylateddimer)
D: Nuclear (gene) linked receptor
Lipophilc ligand (estradiol, vit.A, vit.D, progesterone, T3, NO)
Cytosolic receptor ("nuclear receptor")
Transport to nucleus
DNA binding domain
"response element"
Regulation of transcription
DNA
Transcription-activating domain
Slow response (hours)

Figure 4.8 Types of receptors and signaling mechanisms.
(A) Ion channels receptors. Ion channels inhibitors or ligand-gated receptors (ionotropic receptors) are proteins forming on aqueous pores in the plasma membrane. Ligand binds at a specific site on the receptor. This leads to conformational changes, which open the channel and allow ions to flow into the cell. The change in ion concentration within the cell triggers cellular response. These receptors react in milliseconds. This type of receptor is not to be confused with voltage-gated ion channels that are not considered to be receptors (no signal transduction).
(B) G-protein-coupled receptors. G-protein-coupled receptors (GPCR) are single polypeptide chains forming seven transmembrane domains within the membrane with a segment able to interact with G-proteins.
B1 (inactive state): G-protein (GP) is attached to the cytoplasmic side of the membrane and is inactive as long as a ligand binds to GPCR. An ion channel or enzymes are possible terminal effectors of GPCR.
B2 (active state) steps 1 to 4:
Step 1: binding of a ligand changes the shape of the GPCR that interacts with a GP.
Step 2: interaction causes activation of GP, with guanosine diphosphate (GDP) being exchanged against guanosine triphosphate (GTP).
Step 3: the activated GP (Gα-GTP) moves either towards an enzyme, or directly modulates the conductance of an ionic channel (e.g., muscarinc receptor).
Step 4: when an enzyme is activated (adenylyl cyclase, guanylyl cyclase, etc.) a second messenger (Ca^{2+}, cAMP, cGMP, etc.) is generated and can mediate an action. Then G-protein hydrolyses GTP back to GDP.
The advantage of this signal transduction pathway is twofold: signal amplification and signal specificity.
(C) Tyrosine kinase receptors
C-1 (inactive state): tyrosine kinase receptors (TKR) are transmembrane receptors consisting of individual polypeptides each with a large extracellular binding site and an intracellular tail with an enzyme (tyrosine (Tyr) kinase, serine kinase).
C-2 (active state): when a ligand binds to both receptors, the two receptor polypeptides aggregate to form a dimmer. This activates the tyrosine kinase part of the dimmer. Each uses ATP to phosphorylate the tyrosines on the tail of the other polypeptide as well as other down-stream signaling proteins. The receptor proteins are now recognized by relay proteins inside the cell. Relay proteins bind to the phosphorylated tyrosines and may activate different transduction signals.
(D) Nuclear receptors. Intracellular receptors are located in the cytoplasm or the nucleus (NO, thyroid hormones, steroids, etc.) the signal molecule (lipophilic) must be able to cross the plasma membrane. The ligand binds to a receptor called a nuclear receptor, although some are located in the cytosol (e.g., glucocorticoid receptors) and migrate to the nucleus after binding a ligand. This activation pathway leads to a long-lasting effect, usually over several hours.

glycine receptors are ligand-gated ion channels. The most important veterinary antiparasitic drugs (ivermectins, fipronil) act on ligand-gated anion channels and imidacloprid acts on a ligand-gated cation channel (Raymond and Sattelle, 2002). For ivermectin, glutamate-gated chloride channels have been localized to the pharynx of *Caenorhabditis elegans* and it was suggested that ivermectin could inhibit the pharyngeal pumping in *Haemonchus contortus* that is required for its feeding (Forrester et al., 2004).

Transcription factor receptors such as those for steroid hormones, thyroid hormone, vitamin D, or retinoids receptors are intracellular proteins serving as transcription factors. After binding the ligand, for example a steroid hormone, the activated receptors translocate to the nucleus and bind to a DNA sequence called the *response element* and initiate the transcription of specific gene(s).

Receptor Types and Subtypes

Endogenous neurotransmitters (acetylcholine, norepinephrine, etc.) often bind to more than one type of receptor. This allows the same signaling molecule to produce a variety of effects in different tissues. For example acetylcholine acts via both G-protein receptors (e.g., M_2 muscarinic receptors in the heart) and ligand-gated channels (nicotinic muscle receptors). Historically, the classification of receptors has been based on their effect and relative potency towards selective agonists and antagonists. Now the association of molecular biology and cloning techniques has led to the discovery of novel receptor subtypes and their expression as recombinant proteins has facilitated the discovery of more selective drugs. The International Union of Basic and Clinical Pharmacology (IUPHAR) has a committee on receptor nomenclature and drug classification and regularly publishes updated reviews and a compendium in its Journal (*Pharmacological Reviews*; see www.iuphar.org) (Neubig et al., 2003).

Drug Receptor Theories: from the Occupancy Theories to the Two-State Model

Drug receptor theories consist of a collection of evolving models that correspond to the historical progression of knowledge and permit qualitative and quantitative description of the relationship between drug concentrations and their effect. Before the advent of molecular biology, the various effects of agonists and antagonists were described by operational mathematical modeling, that is with no or minimal mechanistic considerations (a black-box approach). Effects were measured on isolated tissue in an organ bath chamber and the collected data were modeled using relatively simple equations derived from the law of mass action, as in Equation 4.1.

Occupancy Theory was the first model and was proposed by Clark in 1923 (Rang, 2006). The receptor–ligand interaction was described as a bimolecular interaction and the receptor–ligand complex was considered responsible for the generation of an effect. This model assumes that the drug response is a linear function of drug occupancy at the receptor level. Consequently, the drug has to occupy all receptors to achieve a maximal effect (E_{max}), and the response is terminated when the drug dissociates from the receptor. Thus, in the equation for receptor binding (see Equation 4.1) the terms B and B_{max} can be replaced with E (effect) and E_{max}, leading to:

$$Effect = E\max \times \frac{[Drug]}{kD + [Drug]} \tag{4.3}$$

in which *Effect* (the dependent variable) is the observed effect; E_{max} (a parameter) is the maximal possible effect for that system; *Kd* is a parameter measuring affinity and *Drug* is the drug concentration, that is the independent variable. In this simple occupancy model of drug action, the concentration of drug required to produce 50% of the maximal effect (i.e., the EC_{50}) is numerically equal to the dissociation constant (i.e., Kd).

This equation describes the classical hyperbolar agonist–effect relationships. These relationships are often presented on a log scale, in which case they are sigmoidal (see Figure 4.5). It should be noted that some drugs do not follow the classical monotonic dose–effect relationship but rather a U-shaped relationship. Here, drugs may cause low-dose simulation and high-dose inhibition of response (or the inverse) leading to the concept of *hormesis* (for details see (Calabrese and Baldwin, 2003), a challenging concept in toxicology.

The occupation model of Clark was only confirmed in a limited number of cases. As a rule, the physiological response produced by a ligand is not directly proportional to occupancy and it was evident that some drugs acting at the same receptor could elicit different maximal effects at maximal receptor occupancy leading to the notion of *partial agonist versus full agonist*. To account for these discrepancies, which could not be explained by the occupation theory, Ariens and Simonis, (1954) suggested the existence of a proportionality factor, termed the *intrinsic activity*, between the amount of ligand–receptor complex and the observed effect. At almost the same time, Stephenson (1956) introduced the terms of *affinity* for the binding step and the concept of (molecular) *efficacy* for the production of a response. In this concept, a maximal response did not necessarily correspond to 100% receptor occupancy but could occur when only a few receptors were occupied. This led to the concept of *spare receptors* or a *receptor reserve*; thus activation of fewer than 1% of receptors at the skeletal neuromuscular junction, for example, is enough to elicit an action potential and maximal contraction of muscle fiber. According to Stephenson's concept, the final effect of a drug is not directly proportional to the number of receptor–ligand complexes but is linked to the generation of an intermediary step termed *stimulus*, and it is the stimulus that is proportional to the amount of receptor–ligand complex. Finally, the stimulus is translated by the tissue into a more or less amplified response. Thus the efficacy of an agonist, according to Stephenson, is the parameter that indicates its ability to generate the stimulus instead of the final response in Ariens' theory. These concepts of intrinsic activity and efficacy have been historically important to our understanding of the mechanism of drug action because they acknowledge two different properties of molecules: the affinity of the ligand for the receptor (as measured by its Kd) that determines the receptor occupancy, and a second property that is the ability to activate the receptor once bound. Thus a partial agonist may show higher affinity than a full agonist but be less effective in generating a biological response because its intrinsic activity is lower than that of a full agonist (Figure 4.6). Currently, with the advent of molecular biology and the availability of recombinant receptor systems, it is possible to better understand how an agonist actually works and physiologically based models rather than the black-box approach are used to explain the concept of efficacy. Currently, the most generic model is known as the *two-state* model (Figure 4.9).

The two-state model of drug action is consistent with most observations of agonists and antagonists and is genuinely able to explain the nature of inverse agonists and the existence of a spontaneous (constitutive) activity of receptors. Briefly, this model assumes that the receptor molecule exists in two extreme conformations with the active and inactive forms in dynamic

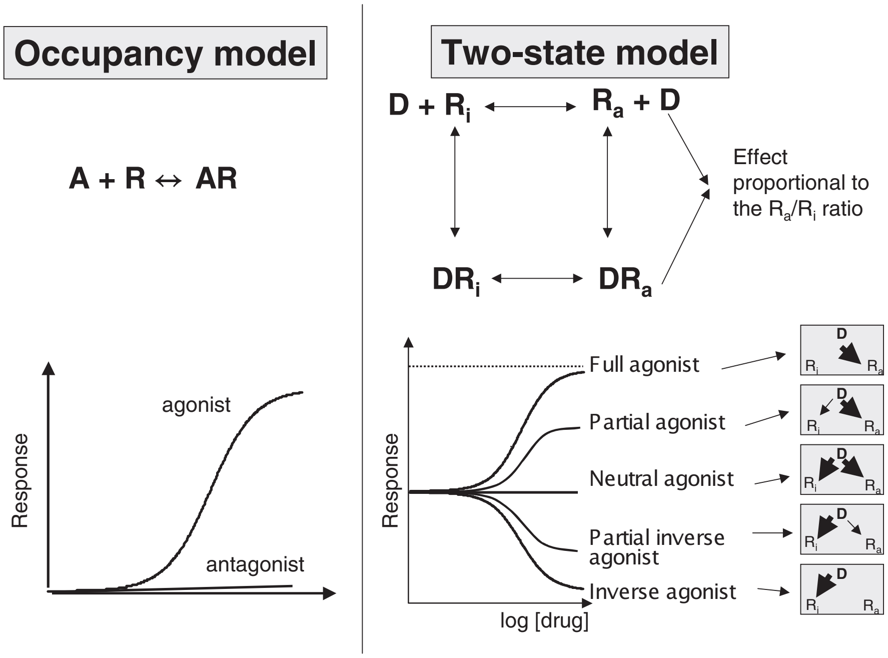


Figure 4.9 Model of receptor activation: the graded activation model (left) versus the simple two-state model (right). The graded activation model implies that agonists can induce different degrees of conformational changes in the receptor (R) and thus different levels of response. In this model, the unliganted receptor is silent (no basal activity) while in the two-state model there is a preexisting resting and an active state. For the occupancy model, the level of response is explained by the concept of efficacy (see Figure 4.3) and an antagonist is a drug with a null (molecular) efficacy but that blocks access to the receptor of other ligands. In the two-state model, a receptor can switch between two conformational states that are in equilibrium: an active state (Ra) and an inactive state (Ri). When no drug (D) is present, a constitutive basal signal output (the existing physiological or biochemical response) is more or less high depending on the actual Ra/Ri ratio. Drug binding to R may shift the equilibrium in either direction. The extent to which the equilibrium is shifted towards Ra or Ri is determined by the relative affinity of the drug for the two conformational states. A drug with a higher affinity for Ra than for Ri will drive the equilibrium to Ra and thereby activate the receptor. Such a drug is called an agonist and a full agonist is a drug selective enough regarding Ra to drive all the receptor in its active state and get a maximal response. When a drug has only a moderately greater affinity for Ra than for Ri, its effect will be lower than for a full agonist and such a drug is termed a partial agonist because it cannot produce a full effect. A drug that binds Ra and Ri with equal affinity will not alter the equilibrium between Ra and Ri, will have no net effect of its own, and will act as a competitive antagonist. Finally, a drug with a higher affinity for Ri than for Ra will shift the equilibrium towards Ri and will produce an effect opposite to that of an agonist and is termed an inverse agonist. For example, cimetidine and ranitidine are inverse agonists of H_2-receptors. Under conditions where the conformational equilibrium of the unliganted receptors is strongly in favor of Ri (i.e., no constitutional spontaneous activity), the model is operationally equivalent to the graded model.

equilibrium. It is the conformational change (i.e., isomerization) of the receptors from an inactive to active state that initiates the pharmacological response. This spontaneous equilibrium may be shifted by the binding of ligands to the receptor. For example, the GABA–benzodiazepine receptor exists in two conformations, an active open channel conformation with high affinity for GABA, and an inactive closed conformation with low affinity for GABA. The two forms are in equilibrium. In this scheme diazepam (an agonist) shows high affinity for the active conformation stabilizing the binding of GABA to the activated conformation. The inactive conformation would be favored in the presence of inverse agonists.

In the framework of this model, an agonist is a ligand that preferentially binds to the active state of the receptor and thus shifts the equilibrium in the direction of the active state. A full agonist is selective for the active form whereas a partial agonist has only a slightly higher affinity for the active receptor than for the inactive form. An inverse agonist is a ligand for which a response may be observed only if there is a preexisting level of receptor activity, as with $GABA_A$ receptors. The two main sources of tone result from either the presence of endogenous ligand or from constitutive receptor activity. These active receptors display a basal effect even if there are no agonist ligands binding to them. A decrease in physiological or biochemical response may be observed if an inverse agonist with a preference for the inactive receptor is added.

In the framework of this biologically based theory, the classical receptor theory has to be revisited. For instance, the measured Kd no longer represents the affinity for either active or inactive receptor but lies in between and Kd is a function of both affinity and efficacy (for details see Rang, 2006). Many compounds previously identified as antagonists appear to act as (partial) inverse agonists, that is they actually diminish the constitutive activity of the receptor. This is the case for cimetidine, which was previously considered an H_2 antagonist. Although the therapeutic relevance of inverse agonists needs to be confirmed, the ligand of choice may be an inverse agonist for some receptors with high constitutional activity. Finally, the last type of ligand to be considered in the two-state model paradigm is the neutral competitive antagonist that has equal affinity for both conformations and does not affect the equilibrium.

The two-state model has given rise to more advanced models such as the ternary complex model or the extended ternary complex model to take into account our knowledge of the signal transduction associated with G-protein-coupled receptors.

Currently, our concepts are still evolving and new descriptive terms such as functional selectivity, agonist-directed trafficking, biased agonism, protean agonism, etc. are being introduced to explain what were previously viewed as artifacts (see Urban et al., 2007; Kenakin, 2008).

Down- and Up-Regulation

The effect of a drug often diminishes when it is given repeatedly. The term used to describe a gradual decrease in responsiveness to chronic drug administration (days, months) is *tolerance*. *Tachyphylaxis* is an acute form of tolerance. Several pharmacodynamic mechanisms (desensitization, loss of receptor, exhaustion of mediator, etc.) and pharmacokinetic mechanisms (metabolism induction, active extrusion of the drug, etc.) may explain tachyphylaxis and tolerance. For loop diuretics such as furosemide, it was observed during a chronic treatment a progressive diminution of maximal natriuretic effect. This tolerance phenomenon is known for diuretics as the *braking phenomenon* and results from activation of the renin–angiotensin–aldosterone system and sympathetic nervous system.

Chronic stimulation of receptors with a drug results in a state of long-term desensitization, also termed *down-regulation*. This is often due to a decrease in the number of receptors, whereas under-stimulation leads to *up-regulation* due to an increase in the number of receptors and a functional supersensitivity. Receptor expression is a dynamic process with equilibrium between the synthesis and destruction of receptors. For example, the binding of a hormone such as insulin to its receptors on the surface of a cell initiates endocytosis of the hormone–receptor complex and its destruction by intracellular lysosomal enzymes. This internalization regulates the number of sites that are available for binding on the cell surface. Although receptor desensitization is generally an unwanted effect, it can provide a way of controlling certain physiological systems. For example, long-term contraception by means of gonadotropin releasing hormone (GnRH) agonist-induced down-regulation of pituitary secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), as deslorelin acetate is now used in dogs and cats. The advantage of this nonsurgical method is its reversibility when the treatment is discontinued.

From the Pharmacological Target to the Animal and Population Pharmacodynamics

Drug *action*(s) (e.g., inhibition of cyclooxygenase) will lead to a pharmacological *effect* (e.g., inhibition of prostaglandin synthesis), which in turn leads to the expected clinical *response* (e.g., reduction of inflammation) and finally to the ultimate clinical outcome (cure or not cure). Drug action, drug effect, clinical response, and the final drug *effectiveness* at the population level are the four main levels of pharmacodynamic description. The clinical response of interest depends on many factors beyond the simple drug action at the enzyme or receptor level. Drug response will be influenced by physiological factors (age, sex, body weight, social behavior, etc.), the genetic background of the animal (breed, strains, etc.), with a possible variability due to polymorphism (see Chapter 50), the disease and its progression (fever, anorexia, etc.), and the treatment itself (previous drug administration, cotreatments, etc.). This explains why the response can vary significantly between animals and, for a given animal, between occasions, making the rational development of a dosage regimen difficult. A dose–effect relationship holds for all drugs, and the goal for determination of a rational dosage regimen is first of a pharmacodynamics nature to specify the range of efficacious plasma concentrations. Indeed, it is a tenet of pharmacology that the plasma concentration is the driving force controlling the biophase concentration and, in equilibrium conditions, the free plasma concentration should be equal, or at least proportional to, the free biophase concentration where receptors and enzymes are located.

The concept of a *therapeutic window* has been coined to describe this range of useful plasma concentrations, in which the expected clinical response is obtained with a minimal risk of adverse effect. The therapeutic window is the optimal range of drug exposure between a low minimally effective concentration and a high toxic concentration. For some drugs the therapeutic window is rather narrow (as for digoxin, bromide, phenobarbital, gentamicin, etc.) requiring possible *therapeutic drug monitoring* to ensure treatment with a reasonable margin of safety. For other drugs, the therapeutic window is large, allowing much more flexibility for the design of a dosage regimen, and these are the only possible drugs suitable for collective treatment or routine prevention (as for most antibiotics and antiparasitic drugs). A concept related to therapeutic window is the *therapeutic index,* which attempts to reflect with a single figure ratio the margin between useful and toxic dose.

An Overview on the Determination of an Effective and Safe Dosage Regimen

The determination of a safe and effective dose or dosing regimen (i.e., dose and dosage interval) is essential for an appropriate exposure of receptors and optimal use of drugs. Dose–response analysis consists of using appropriate scientific tools to answer the following questions (Ruberg, 1995): (i) Is there any drug effect? (ii) Which doses exhibit a different response to the control? (iii) What is the nature (shape) of the dose–response relationship? and finally (iv) What is the optimal dose? In addition, an optimal dosage regimen in veterinary medicine must not only be safe and effective when administered to a single animal in well-controlled conditions but also when administered collectively to food-producing animals. It also needs to be optimized to avoid any public health concerns regarding the questions of drug residues, pollution of the environment, or antimicrobial drug resistance.

Various scientific tools are available to answer these different questions, and the choice of a given approach will be dictated by the therapeutic area, the phase of drug development, the availability or not of surrogate endpoints with good metrological properties, and the ease with which the clinically relevant outcomes can be assessed.

In veterinary medicine the most well-established approach is the so-called dose titration trial. This consists of testing different dose levels and selecting the one that achieves some preestablished regulatory requirement in terms of cure rate (as for antiparasitic drugs).

Although this dose titration approach has its attractions, it also has limitations and these led to the investigation of alternatives such as the PK/PD approach. The plasma concentration profile is generally the driving force controlling the time course of drug concentration at the site of action (the biophase for systemically acting drugs). This led to integration of the PK and PD information by means of a link model, and production of a versatile tool that could be used to determine the optimal dosage regimen. In PK/PD, the variable selected to explore the dose–effect relationship is the plasma concentration profile (not the nominal administered dose).

PK/PD modeling enables the numerical values of the three key PD parameters characterizing any drug, namely its maximal efficacy (E_{max}), potency (EC_{50}), and sensitivity, to be estimated in vivo. The sensitivity is assessed from the slope of the concentration–effect relationship and is a major determinant of the drug's selectivity (see Figure 4.5). Once these different PD parameters have been established, the dose–effect relationship and the time-to-effect relationships corresponding to different possible PK profiles can easily be explored by simulations to predict the time course of drug effects under different physiological and pathological conditions. These different scenarios may then be given to clinicians to assist them in designing future confirmatory clinical trials.

Designs for Dose Titration and the Determination of an Effective Dose

The selection of a dose should be based on well-controlled studies and use accepted statistical methodology. A simple parallel design is often selected in veterinary medicine. In this case the animals (healthy, spontaneously or experimentally infected or infested, etc.) are randomly allocated to a few predetermined dose groups (often including a placebo, low dose, medium dose, and

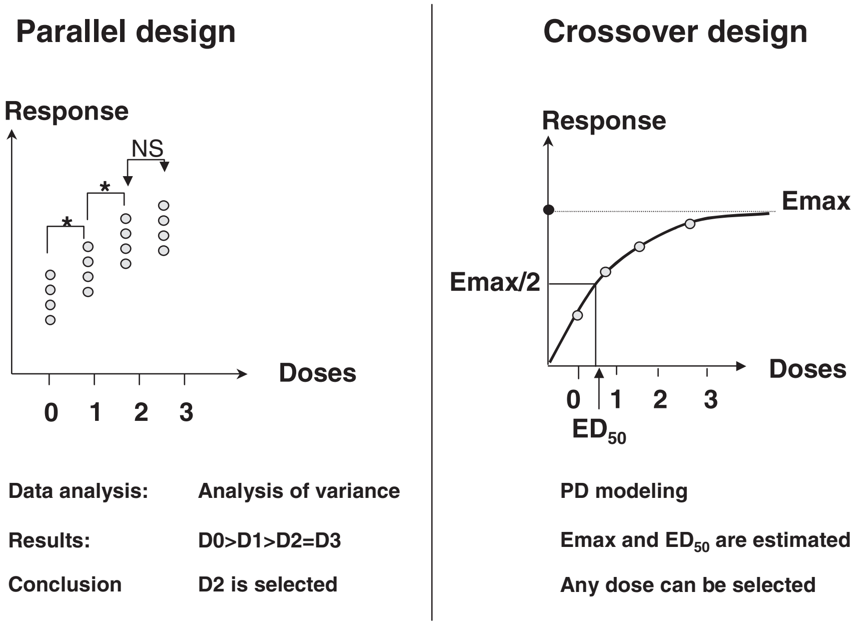


Figure 4.10 Parallel versus crossover design for a dose-titration study. In a parallel design the animals (here n = 4 per group) are randomly assigned to one of the tested doses (0, 1, 2, or 3). Data analysis is performed by a test of the hypothesis (ANOVA), the selected dose being one of the tested doses (no interpolation). Here D2 would be selected because it gives a significantly higher response (∗) than D1 but is not significantly (NS) different from D3. In a crossover design, all the animals receive all the tested doses and individual dose–effect curves are generated. For each individual curve, PD parameters (E_{max}, ED_{50}) can be computed and any dose over the tested range can be selected. With a crossover design, but not in a parallel design, information is obtained about the shape of the dose–response relationship and on variability within the population.

high dose, i.e., four dose levels) and the endpoints of interest are compared using an appropriate statistical test of the hypothesis (Figure 4.10). The parallel design has the advantage of being straightforward in design and analysis but has several serious limits. Since each subject receives only one dose level, it provides only a group mean (population average) dose response, not an individual dose response. This design leads to biased parameter estimates, poor or no estimates of variability, and is unable to provide information on the shape of the individual dose–response relationship (Verotta et al., 1989), which is needed to determine drug selectivity. More importantly, the dose selected as the most effective dose is unlikely to be the optimal dose. The selected dose is obliged to be one of the tested doses and is highly dependent on the power of the experiment's design (number of tested animals), so that trials with a small sample size generally lead to the selection of an excessively high dose.

The parallel design is used in studies of antiparasitic and antimicrobial drugs as the pivotal outcome is eradication of the pathogen (an irreversible drug effect). In contrast, drugs acting on physiological systems (e.g., cardiovascular, nervous system, kidney, etc.) almost invariably act reversibly so that a crossover design becomes possible.

In a crossover design each animal is tested with a range of dose levels so that individual dose–response curves are generated. So long as the number of tested doses is sufficient, the dose–effect relationship for a given animal can then be modeled using a classical sigmoidal relationship of the form:

$$Effect = E_0 \pm \frac{E\max \times Dose^n}{ED_{50}^n + Dose^n} \tag{4.4}$$

where *Effect* (dependent variable) is the predicted effect of a given dose (independent variable) and E_0 the effect without the drug (i.e., a placebo effect or the base line response of the system). E_{max} is the maximum possible effect and ED_{50} is the dose producing half E_{max}. E_{max} is a measure of the drug (physiological) efficacy whereas ED_{50} is a measure of the drug potency (Figure 4.10). It should be stressed that although the two terms efficacy and potency are often confused and used interchangeably within scientific and pharmaceutical communities, the most potent drug may not always offer the most clinically effective dose (see Figure 4.6).

When an exponent (*n*) is included in the model, it reflects the slope of the dose–effect relationship and can provide information about drug selectivity for the tested effect (Figure 4.11).

This relationship can then be used to define the minimum effective dose, the maximum effective dose, and the maximum tolerated dose. The fundamental difference between parallel and crossover designs is that with a crossover design, any dose within the range of doses tested can be selected as the optimal dose because interpolation is possible, whereas this is not the case for a parallel design (see Sheiner et al., 1991 for details).

The Difference Between Dose Titration and the PK/PD Modeling Approach in Determining a Dose

It should be noted that in the following equation, ED_{50} is not a true PD parameter but a hybrid PK/PD variable. In fact ED_{50} is the product of three separate determinants, as indicated by the following relationship:

$$ED_{50} = \frac{Clearance \times EC_{50}}{Bioavailability} \tag{4.5}$$

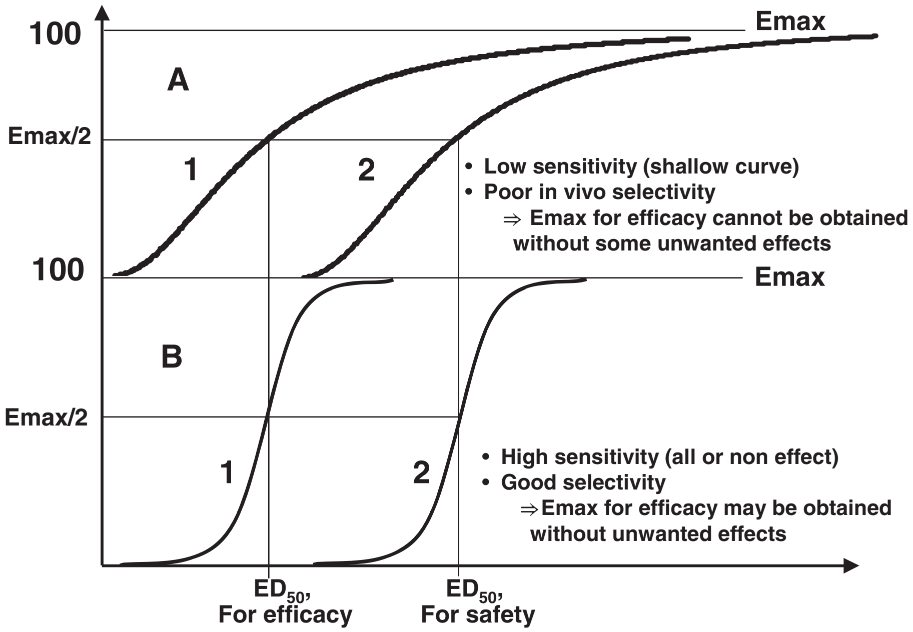


Figure 4.11 In vivo drug selectivity is related to both the therapeutic index (i.e., the ratio $ED_{50,safety}/ED_{50,efficacy}$) and to the slope of the concentration effect relationship. Drugs A (top) and B (bottom) have the same potency (ED_{50}) and the same efficacy (same E_{max}) for the desired effect (curve 1) and unwanted effects (curve 2), that is the same therapeutic index. However, they differ in terms of sensitivity (slope) (shallow for drug A and steep for drug B). Only with drug B, can the full effect (i.e., E_{max}) be obtained without any significant side effect despite the fact that drugs A and B have the same $ED_{50,safety}/ED_{50,efficacy}$ ratio. Definition of therapeutic index by $ED_{10,safety}/ED_{90,efficacy}$ takes into account the differences in sensitivity of dose–response curves.

where *Clearance* is the plasma clearance, *Bioavailability* is the extent of the systemic bioavailability (for the extravascular route of drug administration) and EC_{50} is the plasma concentration providing half E_{max}.

Figure 4.1 illustrates the fundamental differences between a dose-ranging trial and a PK/PD trial. Both aim at documenting the same relationship between dose and drug response but in a PK/PD analysis, the effect is explained by replacing dose with the plasma concentration profile so that the estimated drug potency is expressed as an EC_{50}, not an ED_{50}. The EC_{50}, unlike ED_{50}, is a true PD parameter. There is only one EC_{50} for a given endpoint and this EC_{50} value is not influenced by PK parameters, administration route, or formulation. This means that if a new formulation of the drug is to be developed, there is no need to run a new PK/PD trial. All that is required is a PK study to establish the influence of the new bioavailability on the effect. Hence, EC_{50} has a much wider application than ED_{50}, and the determination of EC_{50} is a primary objective of PK/PD studies. Furthermore, because the plasma concentration–time data include temporal information, PK/PD modeling is appropriate not only for establishing a suitable dose but also the dosage interval.

Building PK/PD Models

In general, three different models are considered when building a PK/PD model: a PK model transforming the dose into a concentration versus time profile, a link model describing transfer of the drug from the plasma into the biophase, and a PD model relating the biophase concentration to an effect (Holford and Sheiner, 1981).

The PK model is generally a traditional compartmental model and the PK parameters are estimated in the conventional way. The PK model is used to provide concentration data for the PD model.

The plasma concentration profile and the effects are usually not in phase, and plasma concentration cannot directly be incorporated into a PD model. The effect of most drugs lags behind the plasma concentration. This can easily be visualized by plotting the effects (Y axis) against plasma concentrations (X axis). A loop is observed when the data points are in chronological order. This is known as a *hysteresis loop* from the Greek word meaning “coming late” (Figure 4.12). When a hysteresis loop is observed, the cause of the delay must be identified to select the appropriate modeling strategy. When the delay is of PK origin (e.g., slow rate of drug distribution at the biophase, etc.), and when the drug effect is directly related to the drug concentration at the biophase level, a so-called *effect compartment model* can be chosen. A link model is interposed between the PK and PD model to account for the delay. Generally, this consists of describing drug transfer into the biophase by a first order rate constant, which is the parameter of the link model. The first-order rate constant (generally denoted as K_{e0}) can be estimated from the time course of the drug effect (see Holford and Sheiner, 1981 for details).

For most drugs, the combination of drug with its receptor is followed by a cascade of time-dependent biochemical, physiological, and/or pathophysiological events (see Figure 4.8). Thus the delay between plasma concentration and the response is due to the intrinsic temporal responsiveness of the system. Indirect response models are used for this type of response.

Four basic indirect response models have been proposed (Dayneka et al., 1993), based on Equation 4.6,

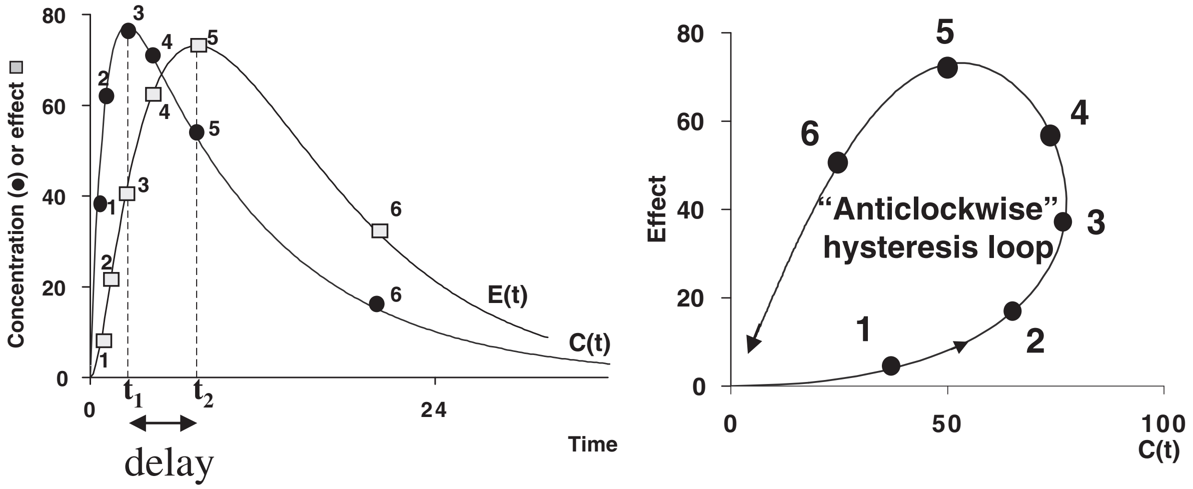


Figure 4.12 In general, plasma concentration time and effect time relationships are not in phase. The peak concentration occurs at time t_1 and peak effect occurs at a later time t_2. An arithmetic plot of effect versus time reveals a hysteresis loop. For any given concentration, two different levels of effect are possible.

which describes the rate of change of response over time with no drug present:

$$\frac{dR}{dt} = K_{in} - K_{out}R \tag{4.6}$$

where *dR/dt* represents the rate of variation in the response variable (R). The model assumes that the measured response is being formed at a constant rate (K_{in}) but disappears in a first-order manner (K_{out}). For the variation in body temperature (°C) during fever, K_{in} (°C per time unit) reflects the level of the thermogenesis and K_{out} (per time unit) expresses the rate of thermolysis. It can then be assumed that the indirect drug action consists of inhibiting or stimulating physiological factors that control production or dissipation of the measured effect, described as:

$$\begin{aligned}\frac{dR}{dt} &= K_{in} \times \{\text{stimulation or inhibition function}\} - K_{out} \\ &\quad \times \{\text{stimulation or inhibition function}\} \times R \end{aligned} \tag{4.7}$$

where the *stimulation or inhibition function* can be the classical $E_{\max}$ model.

The final model to consider in the PK/PD modeling approach is the PD model. There are two main types of PD models, describing either a *graded* concentration–effect relationship or a *quantal* concentration–response relationship. A graded model is used when the response to different drug concentrations can be quantified on a scale (e.g., body temperature, survival time). On the other hand, in a quantal model (also known as a fixed-effect model), the described effects are nominal (categorical) (e.g., dead or alive, cured of parasites or not, appearance of unwanted effects or not). The dose or exposure in quantal dose–response (or exposure–response) relationships is not related to the intensity of the effect but to the frequency of an all-or-none effect. Quantal responses are often clinical endpoint outcomes, whereas graded responses are often surrogates.

The most general model for a graded effect relationship is the Hill model, also known as the sigmoidal $E_{\max}$ model:

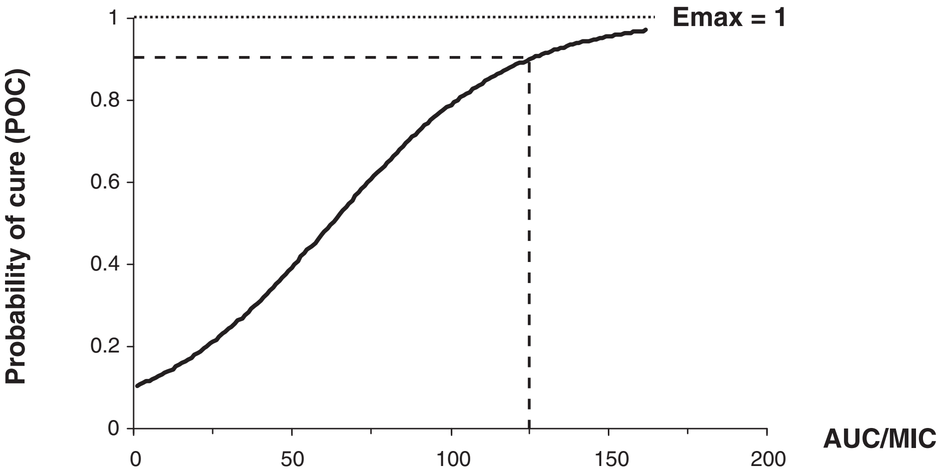
$$E(t) = E_0 \pm \frac{E\max \times C^n(t)}{EC_{50}^n + C^n(t)} \tag{4.8}$$

where *E(t)* is the effect observed for a given concentration at time t (*C(t)*), $E_{\max}$ is the maximal effect attributable to the drug, EC_{50} is the plasma concentration producing 50% of $E_{\max}$, and n is the Hill coefficient, representing the slope of the concentration–effect relationship. When $n = 1$, the Hill model reduces to the $E_{\max}$ model, which corresponds to a hyperbolic function. Many drug effects involve the modulation of a physiological variable (e.g., blood pressure). In this case, inclusion of the term E_0 in Equation 4.8 indicates the presence of a baseline effect. E_0 can also represent a placebo effect.

When the drug effect corresponds to the inhibition of a biological process, the drug effect is subtracted from the baseline (E_0) and EC_{50} is often expressed as an IC_{50}, that is the concentration producing 50% of the maximum inhibition effect ($E_{\max}$). For other PD models see Holford and Sheiner, 1981.

Equation 4.8 contains several parameters (E_0, $E_{\max}$, EC_{50} or IC_{50}, and n). The ultimate goal of PK/PD modeling is to evaluate the means and variances of these parameters from the in vivo observations of *E(t)* obtained over a range of *C(t)* values.

In a graded concentration response relationship, that is when the outcome is binary (e.g., cure or no cure, appearance or no appearance of an adverse effect) or when the



$$POC = \frac{1}{1+e^{2.19-0.03509\times(AUC/MIC)}}$$

Figure 4.13 Logistic regression. When an effect is binary (cure / no cure), a logistic curve is used to describe the relationship between the independent variable (often dose, exposure, or any explicative variable) and the dependent variable (a probability between 0 and 1). This quantal dose–response curve does not relate dose to intensity of effect but to the frequency in a population of individuals in which a drug produces an all or nothing effect. Here, the probability of cure (POC) for a hypothetical concentration-dependent antibiotic has been plotted against the value of a PK/PD index generally selected for this class of antibiotics (i.e., AUC/MIC). The value 2.19 yields P = 0.1 when X is zero (placebo effect) and b = 0.035 gives the "sensitivity," that is the slope of the response. Here, for an AUC/MIC of 125 h, the POC is 0.9 (90%).

results of the drug effect are provided as scores (responder/ nonresponder) the link between the measure of drug concentration exposure and the corresponding probability is given by a logistic equation of the form:

$$P = \frac{1}{1+e^{-(a+bX)}} \tag{4.9}$$

where P is the probability (e.g., of cure from 0 to 1), a is a parameter for baseline (e.g., a placebo effect) and b is an index of sensitivity (similar to a slope), X is the independent variable and may be the exposure (rather than the instantaneous concentration) or a quantitative index predicting efficacy (ex. AUC/MIC for a concentration-dependent antibiotic). Figure 4.13 shows a logistic curve for the probability of cure of an antibiotic. In logistic regression, the dependent variable is termed a *logit*, which is the natural log of the odds, that is:

$$\log(odds) = \text{logit}(P) = Ln\frac{P}{(1-P)} \quad \text{and} \quad odds = \frac{P}{1-P} \tag{4.10}$$

Selection of the Dose and Dosage Regimen

The next step, after estimating all the relevant PD parameters, is to select a dose level and a dosage interval. When the PD parameters have been estimated with a direct effect model and the investigated endpoint has a clinical meaning, Equation 4.5 can be used directly. Often, in the case of NSAIDs, a dose that is close to the ED_{50} is selected and this can easily be computed by incorporating the corresponding EC_{50} into Equation 4.5. For example, in the horse, the estimated potency of phenylbutazone, determined from its effects on stride length in an experimental carpitis, indicated an EC_{50} of 3.6 μg/ml (Toutain et al., 1994). As the plasma clearance of phenylbutazone in horse is 991 ml/kg/day, the calculated dose required to achieve half the maximal effect is 3.6 mg/kg/day. This is slightly below the manufacturer's recommended daily dosage of 4.4 mg/kg. However, if the investigated endpoint for an NSAID is the PGE_2 inhibition, that is a biochemical surrogate, the EC to incorporate into Equation 4.5 may differ from the EC_{50}. It has been shown that at least 90–95% of the magnitude of COX-2 inhibition is clinically relevant. In this case, it is an EC_{95} that should be incorporated in Equation 4.5. In calves treated with tolfenamic acid, for instance, 95% PGE_2 inhibition is obtained with a plasma concentration of 0.245 μg/ml. When this EC_{95} is incorporated into Equation 4.5 together with the plasma clearance of tolfenamic acid in calves (7.2 l/kg/day), a dose of 1.76 mg/kg/24 h is obtained. This is approximately equal to the manufacturer's recommended daily dose of 2 mg/kg.

When the PD parameters have been established using an indirect effect model, the relationship between the

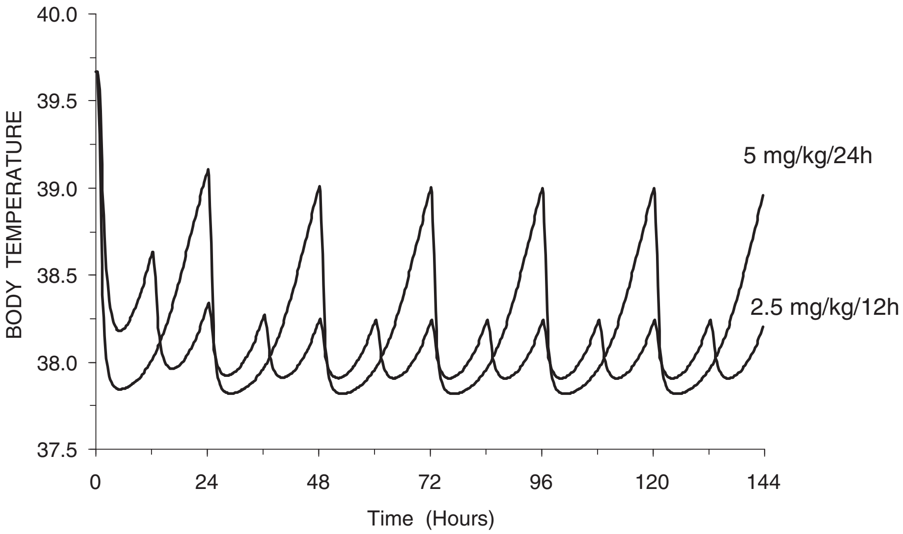


Figure 4.14 Plot of predicted body temperature (°C) versus time (h) after administration of nimesulide in dogs at two different dosage regimens (2.5 mg/kg/12 h and 5 mg/kg/24 h, for 6 consecutive days). Visual inspection of the figure suggests the superiority of the 2.5 mg/kg/12 h dosage regimen. *Source:* Toutain et al., 2001a. Reproduced with permission of John Wiley & Sons, Inc.

clinical response and the EC_{50} is not straightforward. This is also true when the goal is to select the optimal dosage regime to achieve a particular plasma concentration profile (e.g., to maintain plasma concentrations above the MIC for a time-dependent antibiotic) or to determine the most appropriate time interval between two administrations (i.e., to optimize efficacy while minimizing unwanted side effects). In these cases the best approach is to perform simulations. The PK/PD model can be used to simulate a large number of dose and dosage interval scenarios and screen for those dosage regimens with the best efficiency or safety margins. Such analysis requires no additional time or cost during drug development. For example, it was shown for nimesulide in dogs that a PK/PD model predicted a better antipyretic efficacy for nimesulide at a dosage regimen of 2.5 mg/kg twice a day rather than at 5 mg/kg per day, although both dosage regimens were equivalent in terms of lameness suppression (Toutain et al., 2001a) (Figure 4.14).

The PK/PD approach may also be used to determine an optimal dosage regimen for antibiotics. The objectives of rational antibiotic therapy are dual, that is optimization of clinical efficacy and minimization of the selection and spread of resistant pathogens (Lees and Aliabadi, 2002; Toutain et al., 2002). The poor sensitivity of clinical outcomes in indicating the best dosage regimen in terms of bacteriological cure, has prompted investigation of the value of PK/PD surrogate indexes to establish an optimal dosage for antibiotics. Three PK/PD indices appear to be sufficient to predict antibiotic effectiveness: the *f*AUC/MIC ratio, an index used for quinolones; the fC_{max}/MIC ratio (where C_{max} is the maximum free plasma concentration), an index selected for aminoglycosides; and *f*T>MIC (the time during which free plasma concentrations exceed MIC, expressed as a percentage of the dosage interval), an index selected for the so-called time-dependent antibiotics such as β-lactams. All three indices are surrogate markers of what is ultimately expected, that is clinical recovery and bacterial eradication.

It should be noted that these PK/PD predictive indices of in vivo efficacy are built on free (*f or fu*) plasma antibiotic concentrations and not on total plasma concentration or total tissue antibiotic level. Indeed, most pathogens of clinical interest are located extracellularly and the biophase for antibiotics is the extracellular fluid.

Once a PK/PD surrogate index has been selected, its breakpoint values (critical values) need to be considered in relation to the clinical objectives. For quinolones, it is generally reported that the plasma concentration should be five times higher than the limiting MIC (generally the MIC_{90}, i.e., the MIC covering 90% of the bacterial population). This is equivalent to saying that the PK/PD breakpoint, for the index predictive of quinolone efficacy (i.e., AUC/MIC) calculated over 24 h, should be 125 h.

Taking the example of a quinolone, the expected daily dose can easily be computed as:

$$\textit{Daily dose}\text{ for a quinolone} = \frac{\text{Plasma clearance} \times \left(\dfrac{AUC}{MIC}\right)\text{breakpoint value} \times MIC_{90}}{fu \times \text{Bioavailability}} \tag{4.11}$$

where *fu* is the free fraction. The (*AUC/MIC)* breakpoint value in Equation 4.11 is, for example, 125 h and, in this case, the plasma clearance of the quinolone under investigation should be expressed per hour (not per day) if the daily dose has to be computed. Simplified, the daily

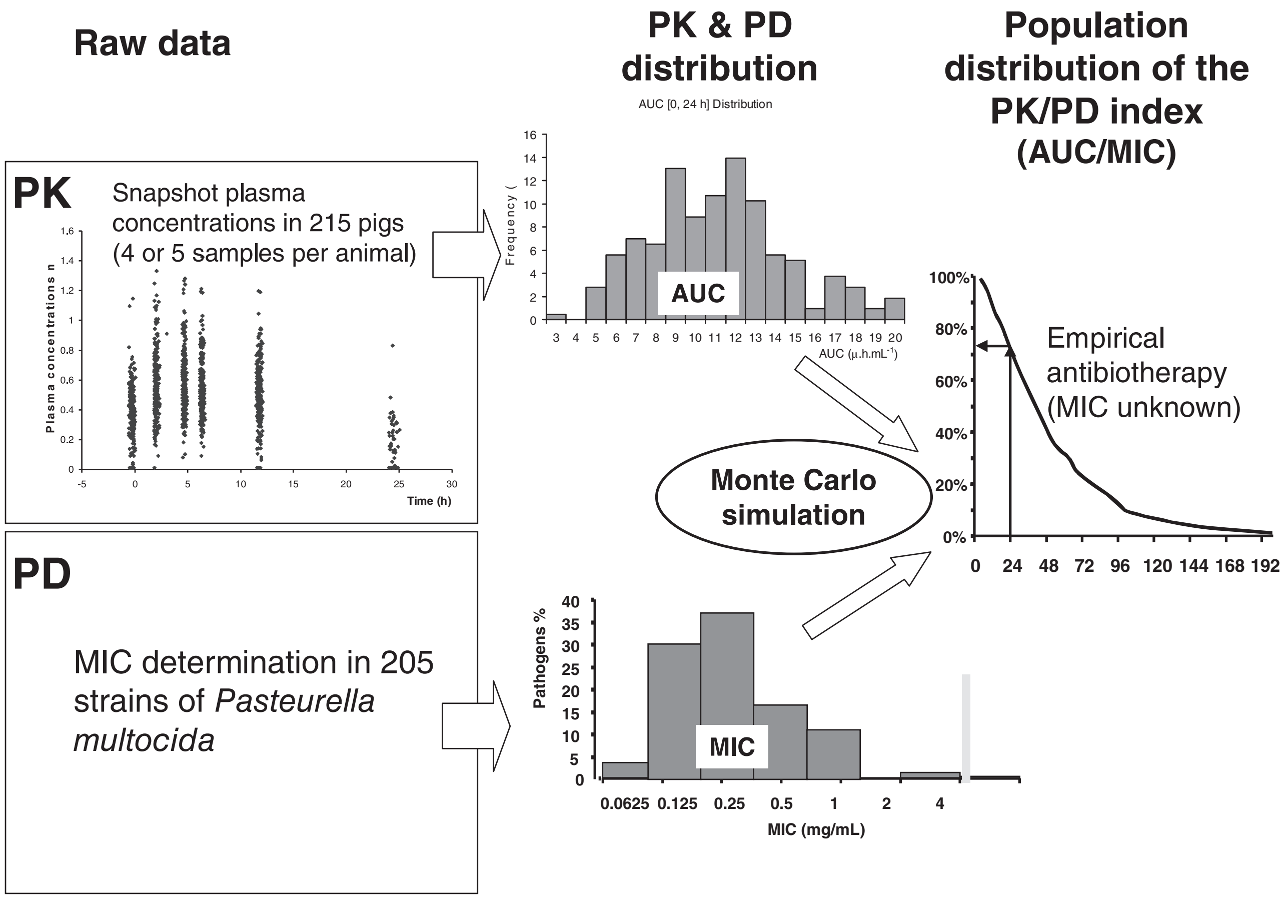


Figure 4.15 Monte Carlo simulation and the PK/PD index population distribution for doxycycline in pigs.
Left top panel: plasma concentrations of doxycycline were measured in 215 pigs under field conditions (4 or 5 samples per pig) after metaphylactic treatment by oral route. The plasma concentrations were obtained with a second doxycycline dose of 5 mg/kg administered approximately 14 h after an initial 5 mg/kg dose.
Left bottom panel: minimum inhibitory concentration (MIC) was determined in 205 strains of *Pasteurella multocida*.
Middle panel: exposure distribution as AUC (0–24 h) and the MIC distribution. These two distributions were used for Monte Carlo simulations. The application of Monte Carlo simulation accounts in a balanced manner for variability in drug exposure (AUC) as well as pathogen susceptibility data (MIC distribution) to establish the population distribution of the relevant PK/PD index (AUC/MIC).
Right panel: the curve gives the percentage of pigs in a population attaining a given value of the PK/PD predictor (AUC/MIC). The curve was generated for an empirical antibiotherapy (MIC distribution known, but MIC for the involved pathogen unknown) based on an administered dose of doxycycline of 10 mg/kg, that is the recommended daily dose regimen. Visual inspection of the curve indicates that a PK/PD index of 24 h (i.e., a daily mean plasma concentration equal to the corresponding MIC) would be attained in 72% of the pigs with an empirical antibiotherapy. If it is assumed that only the free drug is active, the achieved breakpoint should be divided by approximately 10, as the extent of plasma protein binding for doxycycline is about 90%.

dose can be computed using the following equation, which is actually Equation 4.11 with EC_{50} equated to $5 \times MIC_{90}$:

$$\textit{Daily dose} \text{ for a quinolone} = \frac{\text{Plasma clearance} \times 5 \times MIC_{90}}{fu \times \text{Bioavailability}} \tag{4.12}$$

Here, plasma clearance is the daily clearance and 5 the scale factor equivalent to the breakpoint value of 125 h (5 is the round-up of 125 h/24 h) and by which the MIC_{90} should be multiplied to guarantee an appropriate efficacy (for further explanation of Equations 4.11 and 4.12, see Toutain, 2003 and Toutain et al., 2007).

Population PK/PD

One important advance has been to separate the two main sources (PK and PD) of variability through the use of population PK/PD approaches. In this way, population analysis can explain, with some covariates, the variation between animals (or groups of animals) not only in terms of drug exposure but also in terms of drug responsiveness. This is especially true in the case of antibiotics, where clinical response is affected not only by the ability of the drug to reach the site of infection but also by the PD variability (host response to the invading pathogen and bacterial susceptibility). In the case of antibiotics, if both the PK and PD (MIC) variabilities are known from relevant population investigations, the statistical distribution of the selected PK/PD index that is predictive of clinical efficacy can readily be established using *Monte Carlo simulations*. Monte Carlo is a term applied to a numerical method with a built-in random process. It involves combining the variability of antimicrobial drug exposure with the variability in pathogen susceptibility according to the corresponding probability density functions. With Monte Carlo simulations, a large hypothetical population of animals (or outcomes) may be generated to determine the probability of attaining a given PK/PD breakpoint in a given proportion of the population (Figure 4.15). This allows selection of a dosage regimen based on attaining the recommended PK/PD target breakpoint in most animals. For more details see Lees et al. (2006) and, for an example, the case of tulathromycin in the calf (Toutain et al., 2017).

Limits of the PK/PD Modeling Approach for a Dose Determination: Clinical Response versus Surrogate

Very often the drug response of interest is difficult to obtain (e.g., bacterial cure for an antibiotic), difficult to measure quantitatively (e.g., demeanor for a tranquilizer), or delayed in time (e.g., survival time for cancer therapy). Under these conditions, the effect of ultimate interest in a PK/PD trial may be replaced by a surrogate endpoint, that is a biomarker that is objectively measured and validated as an indicator of a normal or a pathological process (Anonymous, 2001; Colburn, 2000). Some examples of surrogates in veterinary medicine are the PK/PD indices that have been proposed for predicting clinical success and bacteriological cure for antibiotics. Angiotensin converting enzyme (ACE) inhibition is used as a surrogate to evaluate ACE inhibitors, although it should be kept in mind that the ultimate goal of therapy with ACE inhibitors is to increase the survival time of diseased animals and the quality of their lives, and not to inhibit an enzymatic activity. Indeed, a drug may have a favorable effect on a biomarker and an unfavorable effect on the disease and any biomarker believed to be a surrogate of clinical relevance needs to be validated. The CAST trial in human medicine is an appropriate illustration (Ruskin, 1989). It was initially thought that pharmacological suppression of premature ventricular contraction (PVCs), identified by postmyocardial infarct monitoring, could reduce the incidence of subsequent arrhythmias. The abilities of different antiarrhythmic drugs to prevent cardiac death (class I antiarrhythmic) were tested against a placebo. It was finally shown that drug therapy was not only associated with a reduction of PVC but also with an increase in the incidence of arrhythmic deaths. This trial illustrates that any surrogate needs to be validated for its clinical relevance even when it has a strong mechanistic link with the condition under treatment.

Conclusion

A drug action is first expressed at a target site (receptor, enzyme, transporter, etc.) where it produces a biochemical or a cellular event such as enzyme inhibition or depolarization. This primary drug action then leads rapidly to some physiological and observable changes such as smooth muscle contraction, a decrease of blood pressure, or an inhibition or stimulation of the secretion of an endogenous substance. This is the primary drug effect. Ultimately these short-time effects are collectively responsible for the delayed overall clinical response such as the survival time or the well-being of the animal. In this context, PK/PD approaches are able to bridge in vitro and in vivo situations, that is to predict the time-development of a drug effect from an in vitro drug action. PK/PD is a tool that is mainly used to select rational dosage regimens (dose, dosing interval) for pivotal confirmatory clinical testing. In PK/PD modeling, the plasma concentration profile (not the dose) plays the role of explicative variable for the effect. Because it separates the two main sources of interspecies variability (PK/PD) it is well suited to multiple-species drug development and interspecies extrapolation. The PK/PD approach offers many advantages over a classical dose titration, especially the possibility of determining a dosage regimen by testing only a single dose. The main limitation of the PK/PD approach is that surrogate biomarkers are used to judge efficacy, that is various drug effects rather than the overall clinical response, the risk being determination of a dose that is effective against the biomarker but does not improve the disease. This is particularly true in chronic disease where the clinical outcome may take months or years to occur.

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5

Principles of Pharmaceutics and Veterinary Dosage Forms

Raafat M. Fahmy and Marilyn N. Martinez

Introduction

Pharmaceutics is an interdisciplinary science that involves the integration of physical pharmacy, pharmaceutical formulation, manufacturing technology, dispensing pharmacy, and the laws of pharmaceutics. Irrespective of whether a drug product is intended for use in humans or veterinary species, the same factors influence the process of dosage form development and quality control considerations. Many of these concepts were introduced in Chapters 2 and 3 of this text and will be expanded upon here to give a perspective on how dosage forms influence drug disposition and activity. These factors include:

- The physical and chemical characteristics of the active pharmaceutical ingredient (API) such as:
  - intrinsic solubility (aqueous solubility of the unionized form of the drug);
  - pKa;
  - ability to traverse the biological membrane;
  - stability:
    - in the presence of excipients
    - during manufacture
    - shelf life
  - crystal characteristics (e.g., crystalline habit, particle size, particle shape).
- The intended use of the dosage form:
  - targeted duration of drug release;
  - stability in the gastrointestinal (GI) tract (orally administered products);
  - ease of administration;
  - limitations on dosage form:
    - size of tablets or capsules or volume of an oral liquid
    - injection volume
    - location of administration (e.g., skin, ophthalmic, ear).
- Formulation that includes excipients to insure that the product delivers the drug in a manner defined by the pharmacologist/clinical expert.
- Method of manufacture (based upon drug and intended dosage form characteristics).

The contents of this chapter provide an overview of each of these points with respect to their relationship to drug products approved for veterinary use. To that end, pharmaceutic principles are discussed from the perspective of the design of drug delivery systems (dosage forms), the formulation and manufacturing variables influencing in vivo and in vitro product performance, and the product understanding necessary to assure that the dosage form is effective, safe, and stable. It should be noted that for those readers interested in learning current recommendations for testing methods for assessing drug intrinsic solubility, the USP has published a general chapter (<1236>) on this topic.

The formulation challenges impacting the animal health pharmaceutical industry parallel those encountered during the development of human pharmaceuticals. In addition, the selection of dosage forms and their corresponding formulations reflect the specific needs of the target animal species, potential breed variation that can influence the rate and extent of drug exposure, the variation in body sizes, species feeding habits, logistical challenges associated with product use, cost to the end user (a question of particular concern for products associated with maintaining herd health), environmental safety, animal disease states (and their impact on drug intake or drug pharmacokinetics), and human food safety (for products intended for administration to food producing animals). To accommodate this diverse range of needs, veterinary medications can be manufactured in a variety of forms including:

- tablets, capsules, and oral solutions and suspensions;
- injectable formulations (solutions, suspensions, and a range of sustained-release formulations);

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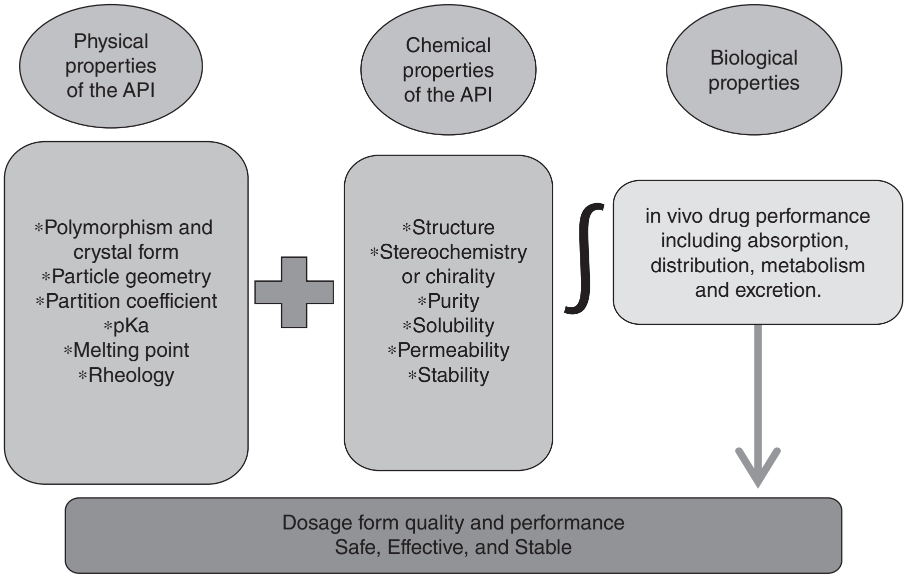
Physical properties of the API
Chemical properties of the API
Biological properties
∗Polymorphism and crystal form
∗Particle geometry
∗Partition coefficient
∗pKa
∗Melting point
∗Rheology
∗Structure
∗Stereochemistry or chirality
∗Purity
∗Solubility
∗Permeability
∗Stability
in vivo drug performance including absorption, distribution, metabolism and excretion.
Dosage form quality and performance
Safe, Effective, and Stable

Figure 5.1 Integration of the physical, chemical, and biological properties of an active pharmaceutical ingredient (API).

- medicated powders for administration in drinking water (herd health);
- drenches (a large volume of aqueous suspension or solution containing the API that is pumped into the animal's rumen);
- medicated articles (a formulation intended for administration in feed. The Type A medicated article is regulated by the FDA as a new animal drug product. However, it is administered to the animal only after substantial further dilution with unmedicated feed);
- topical products (which, in addition to products administered to the skin, includes opthalmics, otics, and intramammary formulations);
- transdermal products (applied to the skin but is systemically absorbed);
- transvaginal products (systemic drug absorption achieved by formulating a product with drug release in the urogenital tract and subsequent absorption across the vaginal epithelium);
- rumen bolus (a large tablet designed for administration in ruminants and which can provide either immediate drug release or remain in the rumen for a prolonged duration of time to provide extended drug release).

Understanding the Active Pharmaceutical Ingredient

Irrespective of the dosage form, it is important to understand the physical and chemical properties of an API prior to product development. Most APIs in use today are solid materials (pure chemical compounds of either crystalline or amorphous structure). In the case of biomass products, the production of the API and related fermentation products need to be carefully controlled.

The purity of the chemical substance is essential for its identification and for determining its chemical, physical, and biological properties. API physicochemical properties may include rheology, particle size, crystalline structure, melting point, and intrinsic solubility, solid-state properties, partition coefficient, pKa, stability, and ionization constant(s) (the extent to which the acid or base ionizes once put into solution). As described in Figure 5.1, integrating our knowledge of the chemical, physical, and biological properties (the latter including the ability of the API to reach its site of action and to generate a biological response) into drug discovery and product development allows for defining the product's Critical Quality Attributes (CQAs) and for formulation optimization.

Physical Properties

Crystalline Form

Many APIs are precipitated during their final stage of synthesis, thereby forming a specific crystalline state. In turn, the crystalline state is a function both of the solvent system used during the initial solubilization process and the conditions of the subsequent drug precipitation. Condition variables of importance include temperature, pressure, rate of cooling, and time. The resulting crystal lattice is comprised of repeating units called unit cells, and all unit cells within a specific crystal are identical (i.e., the same size and arrangement of atoms). Higher levels of energy are required to break strong vs. weak crystal lattices (i.e., characterized by a high melting point). As a result, these more stable crystalline forms typically exhibit low aqueous solubility. Conversely, the amorphous state is characterized by random and irregular positioning of the atoms and a low melting point.

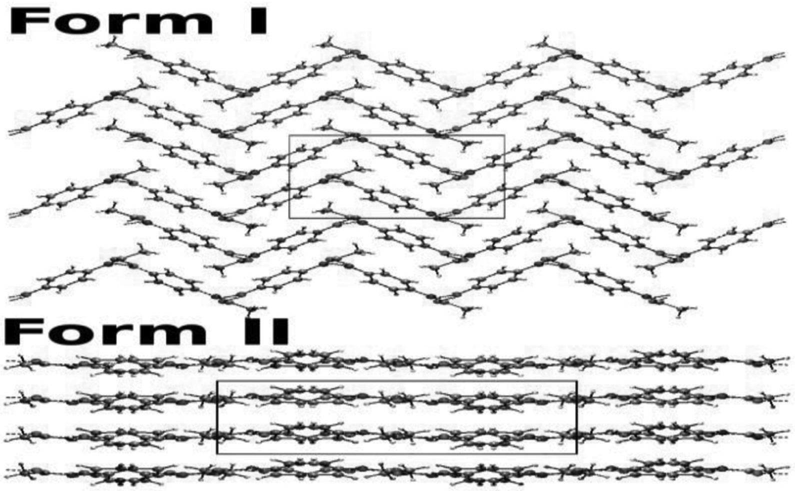

Figure 5.2 An example of two polymorphic forms of the same compound.

An amorphous chemical substance contains no distinct crystalline lattice and therefore is typically the most soluble form of a chemical substance.

Although a low melting point is associated with higher aqueous solubility, it also tends to be less stable. In fact, one of the problems associated with in vivo drug precipitation (solubilized drug that recrystalizes in vivo) is that the administered amorphous form (which was soluble) precipitates into a higher energy form, thereby leading to bioavailability (solubility) problems. It is for this reason that much attention is currently being given to the development of drug formulations that support in vivo supersaturation (i.e., the drug remains in solution at concentrations greater than its thermodynamic equilibrium solubility), thus leading to enhanced intestinal drug absorption (Gao and Shi, 2012).

Polymorphism is a term used to describe the phenomenon where a drug can exist in more than one crystalline form (i.e., with different lattice arrangements). The difference in molecular arrangement is illustrated in Figure 5.2. The resulting crystal lattice can profoundly influence crystal properties. Polymorphs of an API differ in their solubility, dissolution rate, density, hardness, and crystal shape (Sun, 2009).

An additional consideration is that crystalline forms can exist as anhydrous configurations or as hydrates or solvates. Solvates are crystalline solid adducts containing either stoichiometric (fixed integral ratio) or nonstoichiometric amounts of a solvent incorporated within the crystal structure. If the incorporated solvent is water, then the crystalline form is known as a hydrate. The state of molecular hydration can affect solubility. In general, the anhydrous form of the drug is more soluble than the hydrate due to the stability of the crystalline arrangement (i.e., there is a higher melting point associated with hydrates). For example theophylline is available as either the hydrate or as the anhydrous form. Similar differences in polymorphic forms are also seen with penicillin and caffeine. For each of these compounds, the various polymorphs are characterized by differences in their respective in vitro dissolution characteristics, with the solubility and dissolution rate of the anhydrous crystal exceeding that of the hydrate.

Furthermore, the packing geometry leads to differences in their respective lattice energies. Packing geometry is the orientation or conformation of the molecules at the lattice sites, which leads to differences in the amount of energy required to break the crystalline lattice structure. Thus, polymorphic forms usually exhibit different physicochemical properties, including melting point, bulk density, powder flowability, compressibility, hardness, crystal shape, and solubility.

Since at least one-third of all organic compounds exhibit polymorphism, polymorphic screens are an important tool in the pharmaceutical scientist's arsenal. Evaluation of crystal structure, polymorphism, and solvate form is an important preformulation activity. The changes in crystal characteristics can influence bioavailability and chemical and physical stability. Techniques used to determine crystal properties include hot stage microscopy, thermal analysis, infrared spectroscopy, near infrared spectroscopy, and X-ray diffraction.

Particle Geometry

When considering particle geometry, parameters of importance include size and shape. In turn, these influence packing properties and density.

Size: Particle size can affect such CQAs as dissolution rate, content uniformity, taste, texture, color, and stability. The extent to which some of these factors are influenced by particle size is determined, at least in part, by the aqueous solubility of the API (where particle size is more important for the dissolution of poorly soluble compounds) and by the product formulation (for content uniformity). Particle size reduction is usually accomplished by shearing, impact, attrition, or compression (dry-milling processes). An excellent review on the importance of particle size specifications was provided by Sun et al. (2010).

A reduction of particle size increases the particle's surface area, decreases the diffusion (or unstirred) boundary layer (the stagnant layer of solvent surrounding the particle), and therefore may enhance drug solubility. A further discussion of this relationship is provided in this chapter's section on solubility. However, while an increase in surface to volume ratio via a decrease in particle size can enhance API solubility, there are situations whereby decreasing the particle size can have the opposite effect. For example, the cohesivity of particles (tendency to stick together) increases as the particle size decreases. This is a major issue with nanoparticle formulations. Furthermore, particle size governs sedimentation, the formation of aggregates (also known as flocculation, where smaller particles settle faster than do the larger particles) and crystal growth. Crystal growth occurs when smaller particles dissolve and subsequently recrystallize, whereupon they adhere to larger particles, thereby forming larger crystals (a phenomenon referred to as Ostwald ripening). The seeding effect of larger particles on crystal growth is termed nucleation, and a formulation strategy to suppress nucleation is the use of colloid systems (Ravin and Rodebough, 1980).

With regard to manufacturing processes, particle size distribution will affect powder flow. Particles ≥250 μm are usually relatively free flowing while very fine particles (below 10 μm) tend to be extremely cohesive and flow poorly. The converse is true with regard to blend uniformity where smaller particles typically facilitate content uniformity.

Particle shape: Particles can be characterized by their geometric shape, surface characteristics, and whether they are single particles or are associated as multiparticulate bodies. Shape can influence mixing and tableting processes. The simplest way to describe the shape of a particle is to use a metaphor or to compare it to a commonly known object as in such terms as "egg-shaped" and "granular." The following are some of the commonly used descriptors of particle shape:

- *acicular* - slender, needle-like particles of similar width and thickness.
- *flake* - thin, flat particles of similar length and width;
- *plate* - flat particles of similar length and width but with greater thickness than flakes;
- *lath* - long, thin, and blade-like particles;
- *equate* - particles of similar length, width, and thickness;
- *spherical* - globular shaped.

Dissociation Constants/pKa

The pKa of a drug influences lipophilicity, solubility, protein binding, and membrane permeability. Consequently, it affects such PK characteristics as absorption, distribution, metabolism, and excretion (ADME). Typically, the unionized molecule exhibits greater lipid solubility than does its ionized form. Accordingly, the unionized molecule tends to be the form that can most readily cross biological membranes. Conversely, the ionized form is typically responsible for product dissolution in biological fluids (Kerns and Di, 2004; Avdeef, 2001).

Based upon our knowledge of the drug pKa and the surrounding pH, the relative amount of ionized and unionized drug in solution at a particular pH can be determined. The relationship between pKa, pH, and the relative amounts of ionized versus unionized drug can be estimated by applying the Henderson–Hasselbalch (HH) equation introduced in Chapter 2 (Equations 2.2 and 2.3; Figure 2.3). The underpinnings of the HH equation are described by understanding the behavior of weak acids and bases (Figure 5.3).

Strong acids and bases are those that completely dissociate into ions once placed in solution. Weak acids and bases, however, do not fully dissociate in the solution. Weak acids have a higher pH than strong acids because weak acids do not release all of their hydrogens. The acid dissociation constant tells us the extent to which an acid dissociates.

The ionization of a weak acid is represented by (Equation 5.1):

$$HA + H_2O \rightleftarrows H_3O + A- \tag{5.1}$$

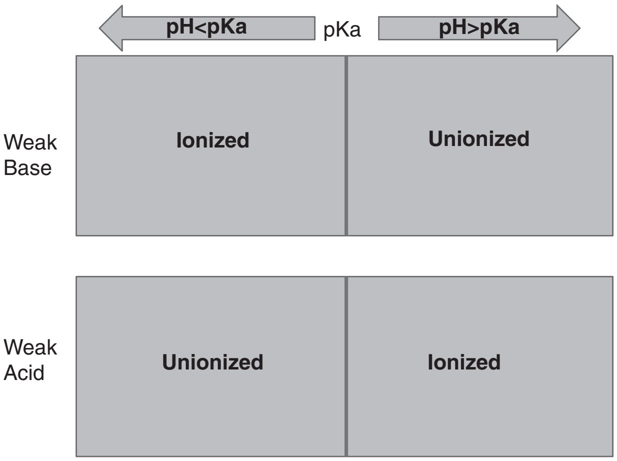

Figure 5.3 The relationship between the preponderance of the ionized versus unionized form of a compound as a function of pKa versus pH for weak acids and weak bases.

The dissociation constant, Ka can be calculated as follows (Equation 5.2):

$$Ka = \frac{(H_3O+)(A-)}{HA} \tag{5.2}$$

The HH equation for a weak acid is described as follows (Equation 3):

$$pH = pKa + log\frac{A-}{HA} \tag{5.3}$$

where A− is the ionized form of the weak acid and HA the unionized form of the drug. Accordingly, when the pH exceeds the pKa, the ionized form of the drug will predominate.

Conversely, for a weak base, pH is defined as follows (Equation 5.4):

$$pH = pKa + \log\frac{B}{BH^+} \tag{5.4}$$

where B is the unionized form of the weak base and BH^+ is the ionized form of the drug. Accordingly, when the pH exceeds the pKa, the unionized form of the drug will predominate.

Based on Equations 5.3 and 5.4, we can calculate the percentage of drug existing in the unionized form at a given pH (Equation 5.5):

$$\%ionized = \frac{100}{1 + 10^{charge(pH-pKa)}} \tag{5.5}$$

where charge = −1 for acids and 1 for bases.

Partition Coefficients

Log P: In most situations, drugs must cross biological membranes before entering the bloodstream. Since biological membranes are lipophilic in nature, there is the competing challenge of solubilization in an aqueous environment versus the need to partition across the lipophilic biological barrier. This fine balance necessitates that the drug can readily pass between the aqueous and lipid phases to insure drug absorption. Figure 2.2 illustrates this concept. This capability is described by the partition coefficient, P, which characterizes the relative oil versus water affinity of a compound when it exists in its unionized form.

The determination of P (or Log P) involves the addition of the API to a separatory funnel that contains a mixture of two immiscible solvents. Drug molecules then distribute between these two layers until equilibrium is established. The resulting estimate, the Log P value, is often used to predict API absorptive capability.

If the compound is acidic or basic, the pH is adjusted to insure the compound is neutralized. Once equilibrium has been achieved, the compound is quantified in each solvent, typically via ultraviolet/visible spectroscopy or by titration, and the logarithm of the concentration ratio is calculated. The measurement is expressed with the following Equation 5.6:

$$\text{Log P} = \text{Log}[(\text{Analyte concentration in Octanol})/(\text{Analyte concentration in water})] \tag{5.6}$$

The resulting Log P value specifies whether the measured compound is hydrophobic or hydrophilic. The reason why octanol is selected as the lipophilic solvent is because it acts both as a hydrogen bond acceptor and donor, thereby simulating the properties of biological membranes (Fletcher et al., 2013).

The interpretation of Log P values relative to permeability predictions are as follow (Lipinski et al., 2001):

- Log P <0 implies that the drug has poor oral absorption. Therefore, parenteral administration may be the preferred method of drug administration.
- Log P = 0–3 implies that the drug has intermediate oral absorption and it can be manufactured as an oral dosage form.
- Log P = 2–4 implies that the drug has high absorption and it can be manufactured as transdermal.
- Log P >4 implies the potential for extensive drug partitioning into fatty tissues, which can lead to drug accumulation.
- Log P>5 implies that the drug exhibits very high lipophilicity and may be unable to traverse across the layers of a biological membrane.

Log D: Another common measure for lipophilicity is the distribution coefficient, Log D. Log D accounts for the existence of molecules both in its ionized and unionized forms. Accordingly, measurements of Log D are generated across a range of pH values. For unionizable compounds, Log P = Log D at any pH. Amongst the different pH values, typically the most interesting is pH 7.4, the physiological pH value.

Melting Point

Each pure substance is characterized by a specific melting point or melting range. The presence of impurities results in a change in the melting point. This phenomenon is commonly used to determine the purity of a drug substance. In addition to its importance regarding the determination of impurities, melting point of the pure API needs to be considered during the manufacturing process. In particular, it can influence the appropriateness of using hot melt extrusion techniques (e.g., chewable dosage forms) or the stability of the product when encountering the heat produced during process granulation.

Rheology

Rheology is a study of the flow and deformation characteristics of a material (liquid or semisolid) when subjected to an applied force. Deformation is the transient change in shape of matter. If the shape of the material reverts to its original state after the stress is removed, the material is said to be elastic (e.g., a rubber ball). Conversely, if the matter does not deform, it is considered to be rigid.

Material flow is dependent on the ability of the material to deform. For example, if toothpaste is placed into a square container and that container is subsequently squeezed, displacing toothpaste into a round container, the toothpaste would take on the shape of the new container. This movement of the toothpaste is defined as flow. Rheologic measurements are used to characterize the ease of flow and the ability of the material to deform. These are important characteristics in the manufacturing process as it influences the ability of the product to maintain its shape after extrusion or the problems that will be encountered during product mixing and storage. The rheological properties of an API can also influence the homogeneity of a formulation.

The ability to deform determines the magnitude of internal friction generated in response to an external stress. In this regard, the greater the deformation properties of the drug, the smaller the friction and the higher the flow rate in response to any given external stress. The measure of resistance to flow is defined as the viscosity of the fluid.

A fluid is considered to exhibit Newtonian flow if the viscosity does not change in response to the application of sheer. An example of a Newtonian fluid is water. In contrast, some non-Newtonian materials (such as yogurt, latex or acrylic paints) exhibit thixotropy (a reduction in viscosity in response to an increase in relative flow velocity by processes such as stirring) while others (e.g., thickening agents) exhibit an increase in viscosity in response to an increase in sheer stress (Wood, 1986).

Chemical Properties

Stability

Stability of the drug substance should be determined in the early phases of product development. A drug product is considered unstable when the API loses sufficient potency that it will adversely affect the safety (e.g., potential for toxic degradants) or efficacy within a timeframe deemed unacceptable for the desired shelf life. Stability testing provides information on the API behavior under a variety of environmental factors such as temperature, humidity, and light. It also establishes a retest period for the drug substance, shelf life for the drug product, and leads to recommendations with respect to drug and drug product storage conditions. Oftentimes, degradation products can be predicted a priori from the chemical structure of the API. For example, esters and amides are sensitive to hydrolytic degradation while acridans and catecholamines are sensitive to oxidative degradation.

Degradation mechanisms include:

Hydrolysis: Hydrolysis is a chemical process that includes degradation of a drug substance by its reaction with water. It typically occurs with esters, amides, and salt of weak acids or strong bases(Alsante et al., 2009).

Oxidation: This process includes the degradation of a drug substance via an electron transfer mechanism, thereby forming reactive anions and cations. Amines, sulfides, and phenols are susceptible to electron transfer oxidation, resulting in *N*-oxides, hydroxylamine, sulfones, and sulfoxide (Gupta et al., 2011). Oxidation can affect the chirality or racemization of some drug substances, leading to altered therapeutic effects (Alsante et al., 2007).

Photolysis: This process includes degradation of a drug substance upon exposure to ultraviolet or fluorescent light. The presence of functional groups such as weak C–H and O–H bonds, nitroaromatic alkenes, aryl chlorides, carbonyls, *N*-oxide, sulfides, and polyenes are responsible for drug photosensitivity. The standard conditions for photostability testing are described in ICH Q1B Photostability Testing of New Drug Substances and Products (ICH, 1996).

Thermal degradation: For some compounds, the addition of heat (thermal energy) can accelerate oxidation, reduction, and hydrolysis reactions.

Humidity: Humidity is a critical determinant of the potential degradants in the finished product (Charde et al., 2013). Chemical stability issues associated with high humidity are generally a result of the hydrolysis of susceptible side chains. High humidity can also facilitate microbial growth. When considering the impact of humidity, it is important to distinguish between free versus bound water. For example, an excipient can contain >10% water but if it is all bound as a crystal hydrate, this moisture will not react with the API. Conversely, if the excipient contains 1% or less of free water, there is a risk that the water associated with that excipient will interact with and alter the API, adversely affecting drug and drug product stability. Drugs containing functional groups such as esters, amides, or lactones are subjected to hydrolysis by water. In these situations, measuring the intrinsic moisture of the formulation is advisable.

Stereochemistry

Stereochemistry is a study of the structural arrangement of the atoms within the molecule or the functional groups in the molecule. Stereoisomers are characterized as having the same composition but differences with respect to its spatial orientation. There are two kinds of stereoisomers: enantiomers and diastereomers. Enantiomers are non-superimposable mirror images. In contrast, diastereomerism occurs when two or more stereoisomers of a compound have different configurations at their stereocenters and are not mirror images of each other. Diastereomers generally have different physicochemical characteristics and therefore are considered to be different compounds.

Whether to formulate a product using a racemate or an enantiomer of a chiral drug (i.e., a drug containing a tetrahedron that results in the presence of molecular forms that are nonsuperimposable mirror images) depends on such considerations as the relative pharmacodynamic activity and the potential toxicity (or side effects) of the individual enantiomers, their pharmacokinetic profiles, and, importantly, the proportions of the individual enantiomers that may form over time in the target animal species (i.e., in vivo chiral inversion) (Baggot, 2007). Furthermore, there can be occasions when one enantiomer of an API is active but the other is not. Use of the racemic mixture leads to half of the administered API being without clinical effect. An example of this is omeprazole versus esomeprazole (Andersson, 2004).

The stereochemistry of the API can also lead to differences in the product dissolution rates. For example, cellulose derivatives, ascorbic acid, mannitol, sorbitol, lactic acid, and malic acid, are examples of chiral excipients that can interact with drug enantiomers and produce stereospecific release and absorption from the formulation.

Geometrical Configuration

Enantiomers are considered to be the same compound but are designated with regard to its geometric configuration. Generally, this is the designation of R or S, reflecting the orientation of each chiral center. R and S forms of a compound can have different biological activities. For example, biological differences due to enantioselectivity are provided in Table 5.1.

An additional geometrical term used to describe the entire molecule is dextrorotatory (D) or levorotatory (L), depending on its exact substituents. D and L symbols are associated with absolute configuration of a molecule, and this designation is based on the absolute configuration of the dextrotatory and levorotatory forms of glyceraldehyde. The D/L system remains in common use in certain areas of biochemistry, such as amino acid and carbohydrate chemistry. Most naturally occurring carbohydrates are nearly all D while most amino acids are L.

Table 5.1 Biological differences between S and R forms of various active pharmaceutical ingredients

API	S Form	R Form
Asparagine	(S) Bitter Taste	(R) Sweet Taste
Chloramphenicol	(S,S) Inactive	(R,R) Antimicrobial
Propranolol	(S) 100 × activity of (R)	
Ethambutol	(S,S) Tuberculostatic	(R,R) Cause blindness
Etodolac	(S) Antiinflammatory	(R) Reduced activity
Verapamil	(S) Cardiovascular effect	(R) Cancer therapy

Optical Rotation

An enantiomer can be named by the direction in which it rotates the plane of polarized light. If it rotates the light clockwise (as seen by a viewer towards whom the light is traveling), that enantiomer is labeled (+). Its mirror image is labeled (−). The (+) and (−) isomers have also been termed d- and l-, respectively (for dextrorotatory and levorotatory). Naming with d- and l- is easy to confuse with D- and L- labeling and is therefore strongly discouraged by IUPAC (Klink et al., 2001).

Solubility

Solubility can be defined as the ability of a substance (the solute) to dissolve in a fixed amount of solvent (e.g., water or buffered solution) to form a saturated solution under a specific temperature and pressure. When the solvent is saturated with solute, the concentration of solute is called the saturation concentration. In the literature, the term *saturation concentration* (C_S) is often used interchangeably with the term *solubility*. Intrinsic solubility for an ionizable molecule is defined as the concentration of the unionized molecule in a saturated aqueous solution, existing in a thermodynamic equilibrium at a given temperature (Horter and Dressman, 2001).

In contrast, when an excess amount of solid is inserted into the solvent, under defined temperature and after sufficient stirring time, the amount of dissolved solute may be initially high but then will reduced to its thermodynamically stable value over time. The time it takes for such a reduction in solubilized concentration depends on several factors, and varies from seconds to months. When the obtained saturation concentrations are much greater than the thermodynamically stable values, the term *apparent solubility* is applied. The term apparent solubility refers to the metastable or dynamic solubility of the materials rather than to a thermodynamically stable state (Mosharraf and Nyström, 2003).

The relationships between dose, dissolution characteristics, and drug solubility can be described in Equation 5.7 as follows (Lobenberg and Amidon, 2000).

$$\text{Dissolution number (Dn)} = (3D/r^2) \times (C_S/\rho) \times \langle \text{Tsi} \rangle \tag{5.7}$$

where

D is the diffusivity of the dissolved drug
ρ is the density of the dissolved drug

C_s is the drug solubility
r is the initial radius of the drug particle
<Tsi> is residence time in the small intestine

The corresponding ratio of dose to dissolved drug i.e., the dose number, D_0, is described by:

$$(D_0) = \frac{M/V_0}{C_S} \tag{5.8}$$

where M is the dose of the drug and V_0 is the volume of fluid consumed with the dose.

Within the pharmaceutical sciences, the focus is on aqueous solubility because water is the biologically relevant solvent. However, it should be kept in mind that, as discussed later in this section, the API must cross biological membranes to exert an effect (e.g., enterocyte, endothelial cell, targeted host cell or a pathogen). Therefore, the relative solubility (partitioning capability) between a hydrophilic and hydrophobic solvent (e.g., octanol versus water) is also a critical consideration when developing drug candidates.

Aqueous solubility is controlled by two opposing interactions. Favoring API solubilization is the interaction between a molecule of the API and the solvent. Counteracting aqueous solubilization is the affinity of the solute for itself. It is the greater of these two effects that dictates the maximum amount of drug that will dissolve per unit volume of solvent. Because many of the more recent drugs exhibit very strong self-affinity (low aqueous solubility), much work is currently being conducted on mechanisms to reduce the strength of these internal interactions, thereby enhancing solubility in aqueous media (Delany, 2005). Pharmaceutical techniques explored for achieving this enhanced aqueous solubility include formulation adjustments or molecular modifications (e.g., new salt forms).

Assessment of drug solubility is not necessarily straightforward as there are numerous variables that affect solubility results. We have already discussed some of these factors, including drug pKa, polymorphisms, particle size and shape, chemical purity, and the potential for molecular stability problems when present in its dissolved state. Several software programs are capable of synthesizing information on these variables into solubility and permeability predictions. Such information can be invaluable both for the formulation chemist and for the pharmacologist who seeks to understand how target animal species-specific in vivo conditions can alter drug product dissolution and drug membrane permeability.

For any set of API chemical characteristics, the solubility of the API in aqueous solutions can be influenced by:

- pH of the solvent: This has its greatest effect on a weakly acidic or weakly basic API. Because it is the ionized form of a molecule that exhibits the greatest aqueous solubility, weak acids typically have its highest aqueous solubility at pH values above their pKa. Conversely, weak bases tend to dissolve better at pH values less than that of their pKa. For that reason, pharmaceutical scientists frequently examine drug solubility across a pH range of 1 to 7.5 (i.e., a range typically encountered in monogastric species).
- Buffer composition: Solubility may be different in different buffers even if the medium is maintained at the same pH. This buffer effect is due to the possible formation of a poorly dissociated compound between the API and the counter-ion in the buffer.
- Common ion effect: The presence of a common ion in the solvent (e.g., contributed by the buffer) and the API can decrease the ionization of the API by a mass effect (i.e., accumulation of the counter-ion towards its own solubility limits will cause the counter-ion to exert a concentration gradient favoring the drug molecule to remain in a solid form).
- Ionic strength: The ionic strength of a solution is a measure of the concentration of ions in that solution. The total electrolyte concentration in solution will affect the dissociation or the solubility of different salts. Since the strength of the common ion effect depends on its concentration (by mass effect), the higher the ionic strength the lower will be the solubility of the API. Apart from the presence of a common ion, the API may have an optimal solubility at a certain ionic strength and minimal solubility at another ionic strength.
- Type of additive: Polar compounds like acids, bases, salts, alcohol, and sugar are very soluble in polar solvents like water. However, nonpolar compounds are uncharged (such as many steroids) and often require the presence of additives, including cosolvents and surfactants:
    - Cosolvent: This is the second solvent which is added in small quantities to enhance the solvent power of the primary solvent. Examples include, dimethylsulfoxide (DMSO), propylene glycol, and various alcohols (such as polyethylene glycol).
    - Surfactant: Surfactants are typically organic compounds that contain both a hydrophobic head and a hydrophilic tail. As a result of their structure, they lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. In so doing, the surfactant can act as detergents, wetting agents, emulsifiers, foaming agents, or dispersants. Examples of surfactants include polysorbates, polyoxyethylated castor oil, polyoxyethylated glycerides, lauroyl macroglycerides, and mono- and difatty acid esters of low molecular weight polyethylene glycols (e.g., lipids, surfactants) which are miscible in aqueous fluids, serving to enhance the drug–water interaction.

- Temperature: The effect of temperature will depend upon whether the solubilization process is endothermic (heat absorbing) or exothermic (heat producing). The dissolution of a solid solute and aqueous solvent is generally endothermic. Therefore, an increase in temperature will increase drug solubility. Conversely, when the solute is a gas, its dissolution in water tends to be an exothermic reaction. Accordingly, any increase in temperature will reduce gas–liquid solubility.
- A review of pharmaceutical efforts to enhance drug solubility was provided by Savjani et al., 2012.

Permeability

As discussed by Martinez and Amidon (2002) as well as in Chapter 2 of this text, molecular movement across lipid bilayers, such as those within biological membranes, is extremely complex due to the regional differences in membrane polarity, hydrophobicity, and density. Generally, the bilayer can be divided into four distinct regions. These are:

- the first (outermost) region, which contains a high proportion of water molecules;
- the second region, which has the highest molecular density of all four regions (contains the polar headgroups), contains little or no water, and exerts the greatest barrier to solute diffusion (due to its density characteristics);
- the third region, which contains the highest density of nonpolar tails and is primarily responsible for the limitations in molecular size and shape associated with membrane transport;
- the fourth region is the most hydrophobic region of the membrane, serving as a hydrophobic barrier in membrane transport.

Owing to the anatomy of this complex barrier, drug permeability is not a simply two-step process of solubilization and diffusion. Rather, it represents a spectrum of complex molecular events. Accordingly, membrane penetration reflects the interaction of several factors, including molecular size (negatively correlated), lipophilicity (positively correlated), polar van der Walls surface area (negatively correlated), and the molecular flexibility (intramolecular hydrogen bond formation).

Molecular flexibility and the corresponding ability to undergo conformation changes can significantly affect the polar surface area of a molecule. The polar surface area and nonpolar surface area are predictors of intestinal permeability, being respectively inversely and directly related to membrane permeability. Another important variable is the strength of the hydrogen bonds formed between the molecules of water and solute. It is generally assumed that these bonds must be broken (desolvation) before the solute can traverse the biological membrane.

Impurity can affect stability, appearance, and API toxicity, (e.g., aromatic amines, suspected of being carcinogenic). In most cases, the impurities (reactive species) consist of water, small electrophiles (such as aldehydes, carboxylic acid derivatives, and peroxides), and metals. Water can hydrolyze some drugs. Aldehydes and carboxylic acids can form molecular adducts. Peroxides can oxidize some drug. Metals can catalyze oxidation, hydrolysis, and other degradation pathways (Fahmy et al., 2008). Impurities can lead to altered bioavailability, toxicity, or a reduction in effectiveness.

Formulation Elements

There are more than 800 excipients currently used in marketed pharmaceutical products within the United States (human and veterinary). This number is expected to increase to accommodate the evolution in therapeutic entities such as those associated with gene and cell therapy or the development of new drug delivery platforms (Bhattaryya et al., 2006).

The purpose of excipients varies as a function of the drug, dosage form, and the intended in vivo release characteristics. Pharmaceutical excipients may be used for any one (or combination) of the following objectives:

- aid in the processing of the dosage form during its manufacturing and provide the desired drug delivery characteristics;
- enhance solubility and bioavailability of the active ingredient;
- enhance palatability to some dosage forms (tablets, chewable, suspension, solution, etc.);
- maintain pH and osmolarity of liquid formulation;
- control the drug action inside the body;
- enhance the overall stability of the drug during the shelf life;
- provide physical, chemical, and microbiological stability to the dosage form.

Both synthetic and natural ingredients have been used as excipients in pharmaceutical products. Examples include:

- Natural products: Gelatin, acacia, agar, shellac, starch, gums, beeswax, oils, lanolin, vanilla, coco butter, sugars, and cellulose. Note that some cellulose-based excipients are less frequently used in recent formulations due to their tendency to exhibit lot-to-lot variability and due to their potential to act as substrates encouraging fungal and bacterial growth.
- Semisynthetic products: These provide less variability than natural products, helping the formulator design a consistent product, and include synthetic derivatives

of cellulose (e.g., microcrystalline cellulose, hydroxyethyl cellulose, hydroxyethylmethyl cellulose, cellulose acetate phthalate, hydroxypropyl cellulose).
- Synthetic products: These usually originate from petroleum products or byproducts, typically exhibit consistent physicochemical properties, and do not support any microbial growth. Examples are povidione (PVP), polymethacrylates, polyethylene glycol, polyethylene oxide, and polyvinyl alcohol.

For excipients not described in any pharmacopoeia, specifications should include physical characterization, identification tests, purity test, assay, and impurity tests. As with human pharmaceuticals, all excipients used in the veterinary medications are manufactured and supplied in compliance with compendial standards and should comply with the United States Pharmacopeia (USP) or National Formulary (NF) monographs or with any other recognized pharmacopeia.

The Food and Drug Administration (FDA) approves all excipients that are included in pharmaceutical products. A certification of analysis (COA) must confirm that excipients are of nonanimal origin. If this is not the case, the regulatory agency requires documentation to demonstrate freedom from viral and transmissible spongiform encephalopathies (TSE) and bovine serum encephalopathy (BSE) risks.

Excipients from an animal origin should be identified. The genus, species, country of origin, source (e.g., pancreas), and manufacturer or supplier should be clearly indicated. Furthermore, for excipients derived from ruminant materials, the application should state whether the materials are from BSE countries as defined by the US Department of Agriculture (9 CFR 94.11). Guidance is available from the FDA on *The Sourcing and Processing of Gelatin to Reduce the Potential Risk Posed by Bovine Spongiform Encephalopathy (BSE) in FDA-Regulated Products for Human Use*. The potential adventitious agents should be identified, and general information regarding control of these adventitious agents (e.g., specifications, description of the testing performed, and viral safety data) should be provided in this section. Details of the control strategy and the rationale for the controls should be provided.

Additional guidance is available in International Conference on Harmonization (ICH) guidelines:

ICH: *Q5A Viral Safety Evaluation of Biotechnology Products Derived From Cell Lines of Human or Animal Origin*
ICH: *Q6B Specifications: Test Procedures and Acceptance Criteria for Biotechnological/Biological Products.*

It is critical that the API and excipients do not interact with each other and/or the container over time. If interaction occurs, product quality and performance can be compromised. An additional consideration is the safety of the excipient. The safety of a particular component of human food and drugs does not necessarily insure its safety for consumption by a veterinary species. For example, xylitol, a popular human sugar substitute was found to be highly toxic, or even fatal, when given to dogs.

Some excipients have their own inherent activity that can influence drug pharmacokinetics. For example, the vitamin E derivative, α-tocopherol polyethylene glycol 1000 succinate (TPGS), when administered at low concentrations, appear to enhance the secretion of chylomicrons by enterocytes. In turn, this can lead to a significant enhancement of intestinal lymphatic transport of lipophilic drugs. In addition, several reviews show that a number of excipients can influence the in vivo and in vitro activities of a variety of cytochrome P450s (CYPs) or membrane transporter activities (Ren et al., 2008; Buggins et al., 2007).

For any given dosage form, the excipient is selected to achieve a specific function. While there can be multiple choices of excipients, the final selection is based upon its specific properties and how it will impact product in vivo performance, stability, and ease (and uniformity) within the manufacturing process. In the next section, we provide a brief overview of the functional considerations that go into the determination of excipients included in solid dosage forms.

Excipients Typically Used in Veterinary Dosage Forms

The following are some examples of the common excipients and their functionalities used in manufacturing different veterinary dosage forms.

Excipients for Solid Dosage Forms

Examples of commonly used dosage form-specific excipients are described below.

Fillers (Diluents): Diluents are added to a formulation to increase the bulk volume of the API, making the size of the tablet suitable for handling. They also can improve the product manufacturing properties (e.g., powder flow, compaction properties, and homogeneity) and product quality, such as content uniformity, in vivo disintegration and dissolution, tablet integrity, friability (the ease with which tablets can break into smaller pieces, promoting more rapid dissolution), or physical and chemical stability. The selection of diluents depends on the type of processing and the plasticity (the capability to be deformed) of the materials to be used. Examples of diluents used in veterinary medicine include: microcrystalline cellulose (MCC), lactose, dicalcium phosphate (Di-Cal), mannitol, sucrose, and partial pregelatinized starch.

Binders: Binders are used for the purpose of holding together the ingredients in a formulation, thereby ensuring that granules and tablets can be formed with the required mechanical strength. It also affects the disintegration rate, dissolution rate, hardness, and friability (Jayesh and Manish, 2009). Examples of excipients that are added to a formulation in a solid form include hydroxypropyl methylcellulose, polyvinylpyrrolidone (PVP), starch, and polyethylene glycol. Alternatively, it could be dissolved in solvents and added to the powder to form wet granulation such as gelatin, PVP, starch, sucrose, and polyethylene glycol (PEG).

Disintegrants: Disintegrants are added to promote moisture penetration into the dosage form matrix, thereby ensuring that it will break into small fragments. The formation of smaller tablet particles allows for an API to undergo a more rapid dissolution. Disintegrants can be added to tablets and hard shell capsule formulations (i.e., the drug plus formulation administered as a solid substance enclosed in a capsule). The quality and quantity of the disintegrant affects the product's dissolution and therefore the bioavailability of the API. Examples of common disintegrants used in veterinary medicine are: starch, (MCC), croscarmellose sodium, and crosspovidone.

Glidants: Glidants are used to promote powder flow within the manufacturing machinery by reducing interparticle friction and cohesion. Glidant particles coat the surfaces of the larger bulk powder particles ('host particles') where they may reduce of surface roughness and attractive forces, or act as moisture scavengers. Examples of glidants are: talc powder, sodium lauryl sulfate, colloidal silicon dioxide, and starch.

Lubricants: Lubricants are added to formulations to prevent its sticking to the manufacturing equipment. Although there are both hydrophilic and hydrophobic lubricants, it is the latter that is more effective in achieving the desired manufacturing benefit and therefore is the one generally used in tablet formulations. Commonly used hydrophobic lubricants include magnesium stearate, calcium stearate, and stearic acid. Of particular note for these hydrophobic (waxy) lubricants is that the dissolution rate of poorly soluble APIs is influenced not only by the amount of lubricant added to the formulation but also by the order of its addition relative to the other constituents and the blending time of the lubricant with the other ingredients. This effect appears to be attributable to the smearing of the hydrophobic lubricant (e.g., magnesium stearate) over particle surfaces, resulting in a reduction in product in vivo absorption (Kalyana et al., 2011).

Coating agents: Tablet coating is used to improve product appearance, to provide taste/odor masking, to facilitate swallowing, to protect from moisture/light, to improve mechanical strength, and as a mechanism for modifying drug release (e.g., enteric coated tablets). Examples of coating agents include sugar, hydroxypropyl methylcellulose (HPMC), cellulose acetate phthalate (Aquacoat® CPD; enteric), ethylcellulose (sustained release), hydroxypropyl methylcellulose acetate succinate (enteric), and polyvinyl acetate phthalate (enteric).

Excipients for Modified Release Formulations

Excipients used in modified release (MR) tablets fall into two broad groups based on whether they are cellulose or acrylic polymers. The most common technique for producing a MR tablet is either through the formation of matrices or by the inclusion of special tablet coatings.

Matrix tablets: The most common excipients for the matrix tablets are sodium carboxymethylcellulose, HPMC high viscosity, a carboxyvinyl hydrophilic polymer, and PVP.

Coated tablets The most common excipients for coated tablets are ethyl cellulose (the most frequently used cellulose-based polymer used for extended release) and PVP.

Excipients Typically Used in Liquid and Semisolid Formulations

Bulking agents: These excipients are generally used to increase the mass of a lyophilized product, thereby providing structure to the formulation. Bulking agents are used for low-dose, high-potency drugs (when the API constitutes less than 2% in the formulation) and therefore does not have the bulk necessary to support its own structure. Examples of bulking agents include mannitol, lactose, and sucrose.

Buffering agents: The choice of buffer depends on the pH stability profile of the API. Buffer selection, along with its concentration is important to avoid API degradation during processing or during storage and reconstitution. Buffer selection also influences the optimization of product solubility. Commonly used buffers in the parenteral formulations are acetate, citrate, tartrate, phosphate, triethanolamine (TRIS), benzoate sodium /acid, boric acid/sodium, arginine, hydrochloric acid, sodium hydroxide, succinate sodium, sulfuric acid, and sodium carbonate.

Tonicity adjusting agents: Injectable formulations should preferably be isotonic to prevent osmotic shock at the injection. Examples of commonly used tonicity adjusting agents are alkali metal or earth metal halides such as $CaCl_2$, KBr, KCl, LiCl, NaI, NaBr, NaCl, Na_2SO_4, or boric acid. Nonionic tonicity agents include glycerol, sorbitol, mannitol, propylene glycol, or dextrose.

Preservatives: The typical preservatives used in liquid formulations include a range of antioxidants, antimicrobial agents and chelating agents (Chaubal et al., 2006).

Antimicrobial agents: These are used to stop the growth of any microorganisms in the drug product. Examples include benzyl alcohol, parabens (methyl, propyl, butyl), benzoic acid, phenol, thimerosal, meta-cresol, benzalkonium chloride, chlorobutanol, glutathione, and others.

Chelating agents: Chelating agents (substance that bind with free metals such as Cu^{+}, Cu^{2+}, Fe^{2+}, and Fe^{3+}), are generally permitted to be present in trace amounts in the formulation. Since the newly formed complex ions are nonreactive, chelating agents remove the capacity of the metal catalysts to participate in oxidative reactions. Chelating agents also have the ability to enhance antimicrobial effectiveness by forming a metal-ion–deficient environment that otherwise could feed microbial growth. Examples of chelating agents include sodium calcium, ethylenediaminetetra acetic acid EDTA, diethylenetriaminepenta acetic acid (DTPA), calteridol, and disodium EDTA.

Antioxidants: These are used to prevent or minimize oxidation reactions over the shelf life of the product. The most commonly used antioxidants in sterile formulations are ascorbic acid, acetylcysteine, sulfurous acid salts (bisulfite, metabisulfite), and monothioglyercol.

Solubilizing agents: Water is a common solvent system. However, nonaqueous water-miscible agents are used as cosolvents with water to enhance the solubility, stability, and to control drug release from injectable products. Those solubilizing agents, added to the injectable products to enhance the drug solubility into the formulation, are generally classified as surfactants or cosolvents. In contrast to surfactants, which increase API dissolution by reducing the surface tension of the chemical substances, cosolvents are defined as a second solvent that is added in small quantities in conjunction with the primary solvent to increase the API solubility. For compounds with large hydrophobic groups and high Log P values (e.g., 3–4), the use of cosolvents in combination with surfactants may be required (Pramanick et al., 2013). Examples of surfactants are polyoxyethylene sorbitan monooleate (Tween 80), sorbitan monooleate polyoxyethylene sorbitan monolaurate (Tween 20), lecithin, and polyoxyethylene–polyoxypropylene copolymers (Pluronics). Examples of cosolvents include propylene glycol, glycerin, ethanol, polyethylene glycol (300 and 400), sorbitol, dimethylacetamide, and cremophor EL.

Complexing and dispersing agents: Complexation is sometimes used to improve the solubility of drug in the solvent, especially water. Cyclodextrins have emerged as very effective additives for solubilizing hydrophobic drugs. In the parenteral dosage forms, modified cyclodextrins such as hydroxypropyl-β-cyclodextrin and sulfobutylether-β–cyclodextrin have been reported to be highly efficient solubilizer/stabilizers (Loftsson et al., 1996).

Flocculating/suspending agents: Flocculating agents are used in parenteral suspension injectable products to enhance particle “dispersability,” reduce sedimentation rate in the flocculated suspension, prevent cake formation, and control viscosity. Examples of flocculating agents are potassium/sodium salts such as the chloride, citrate, and acetate (all used primarily in parenteral formulations). Other suspending agents (which may be used in nonparenteral or parenteral formulations) include sodium carboxymethyl cellulose, acacia, gelatin (generally for oral formulations), methyl cellulose, and PVP (Anderson et al., 2006). It should be noted that carboxymethyl cellulose may cause allergic reaction in humans, horses, and cattle (Deweck and Schniede, 1972).

Wetting agents: These excipients are used to create a homogenous dispersion of solid particles in liquid media. The majority of drugs in aqueous suspensions are hydrophobic and are therefore difficult to suspend. Consequently, they tend to float on the surface of any polar liquid due to entrapped air and poor wetting. The inability of particle wetting reflects a high interfacial tension between material and liquid. Consequently, the interfacial tension must be reduced so that air can be displaced by liquid. Wetting agents are surfactants that lower the interfacial tension and contact angle for solid particles and liquid vehicle. Examples of wetting agents include anionic substances (such as sodium lauryl sulfate, sulfonate sodium, and docusate sodium), cationic substances (such as cetylpyridinium chloride), zwitterions (such as lecithin), and nonionic substances (such as poloxamer and polysorbate). It is the nonionic surfactants that are most commonly used as wetting agents in pharmaceutical suspensions.

Thickening agents: Commonly used naturally occurring thickening agents are acacia, agar, cellulose, and gelatin. Synthetic thickening agents include methylcellulose, microcrystalline cellulose, and colloidal silicon dioxide.

Coloring agents: Coloring agents can be classified as soluble in water (dyes) or insoluble in water (pigments). The colors approved for the clear liquid formulations are limited to dyes. Examples include sunset yellow, methyl blue, quinine yellow, and FD&C red.

Humectants: These substances prevent drying or loss of moisture from a product commonly used in semisolid preparations. Examples include glycerin, propylene glycol, and sorbitol.

Flavors These substances are used to conceal or mask the bitter, salty, or offensive taste or odor of a drug substance.

Dosage Form Considerations

Pharmaceutical dosage forms contain both pharmacologically active compounds and excipients. For any given product, its quality and performance will be a function of the physicochemical properties of the API, the excipients, and the manufacturing process.

APIs are formed into dosage-form products before they are dispensed or administered to animals. The API is mixed with excipients, such as binders, fillers, flavoring and bulking agents, preservatives, and antioxidants. These ingredients may be dried, milled, blended, compressed, and granulated to achieve the desired properties before they are manufactured as a final formulation.

Examples of the dosage form classifications commonly found within the framework of veterinary medicine are provided below (Figure 5.4). Each dosage form is tailored to meet the needs of the specific animal species, the disease condition (e.g., intramammary) or therapeutic goal (intervaginal implants for estrus control), conditions of use (e.g., in hospital, at home use, mass herd treatment).

Types of Dosage Forms: Liquids and Semisolids

Solutions: Solutions are a clear, homogeneous liquid dosage form that contains one or more APIs dissolved within a vehicle. Solutions are a stable system (i.e., the molecules or the ions do not settle out). Particles are not visible, even under highest magnification, but the solute itself could differentially absorb visible light, leading to color. Depending upon its intended route of administration, the solution may or may not need to be manufactured as a sterile product. In cases when the API is poorly soluble, the formation as a solution may necessitate the addition of solvents such as alcohol, propylene glycol, or glycerin.

Formulations for oral solutions include solvents, buffers, preservatives, antioxidants, and flavors. Challenges with the manufacture of a solution include API solubility, formulation stability (due to oxidation, pH, temperature, and light), sterility (for some routes), and the potential for microbial contamination. Quality attributes include appearance, pH, assay, impurities, and microbial limit.

Suspensions: A liquid suspension is a dosage form containing solid particles dispersed in a liquid vehicle. Unlike solutions, suspensions are considered to be thermodynamically unstable (i.e., capable of separation). Suspensions are for the administration of insoluble or poor soluble drugs.

The suspension system consists of two phases: the internal phase, which contains the solid particles that are characterized by a specific size distribution; and the external phase (suspending medium), which while often

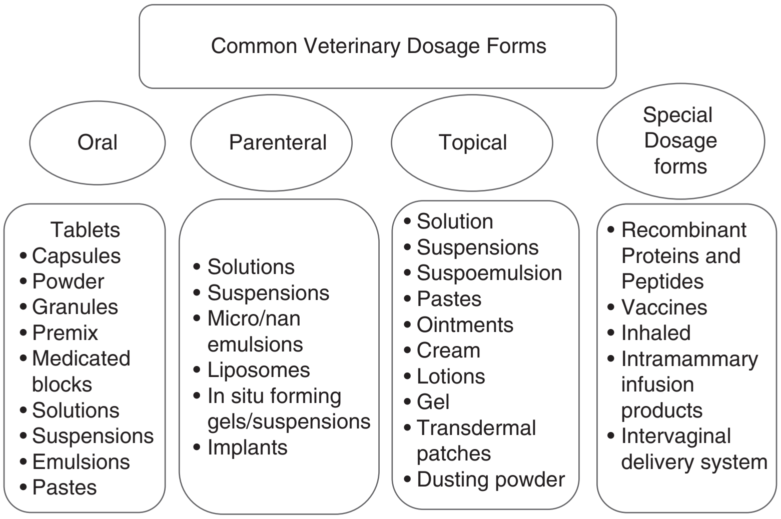

Figure 5.4 Examples of common veterinary dosage forms as a function of route of administration.

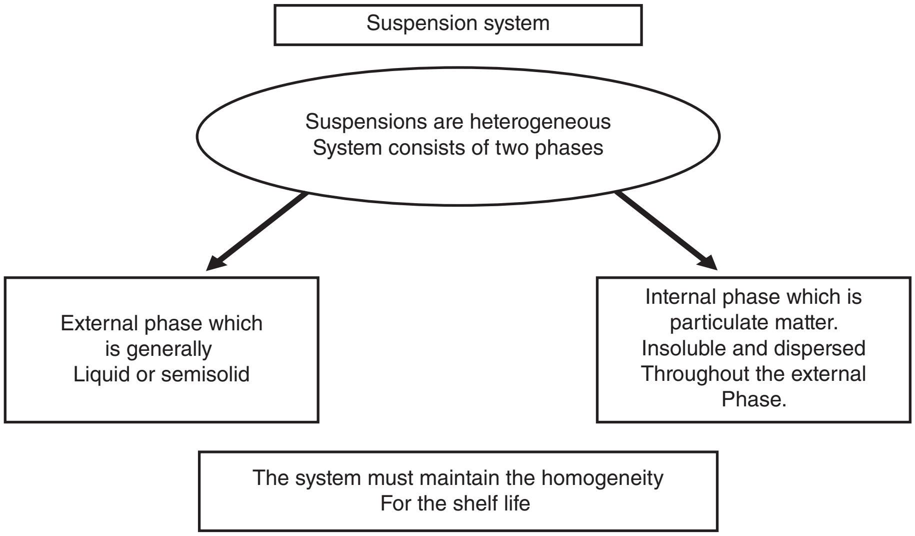
Suspension system
Suspensions are heterogeneous System consists of two phases
External phase which is generally Liquid or semisolid
Internal phase which is particulate matter. Insoluble and dispersed Throughout the external Phase.
The system must maintain the homogeneity For the shelf life

Figure 5.5 Considerations that need to be applied when formulating a drug product as a suspension.

aqueous, can also be organic or oily. This is illustrated in Figure 5.5.

Suspensions can be classified according to their particle size:

- Course suspensions contain particles exceeding 1 μm in diameter.
- Colloidal suspension contain particles less than 1 μm in diameter.
- Nanosuspensions contain particles diameters equal to or less than 100 nm.

There are several challenges encountered during the development of suspensions. These include:

- the electrostatic forces that can lead to particle clumping;
- identification of a vehicle that is compatible with the density of the solid particles. If the density of the particles is greater than the vehicle, the particles will settle downwards (sedimentation). Conversely, if the density of particle is less than that of the liquid medium, particles will move upward (creaming);
- particle size control;
- viscosity, which influences handling of the material during manufacturing and by the end user;
- rate of settlement and resuspendability;
- stability and shelf life.

Formulations for suspension include surfactants, buffers, suspending agent, thickening agents, preservatives, antioxidants, and flavors. When dealing with formulations containing two phases (e.g., solid and liquid), there exists an electrical interactive force. Particles contain an electrical charge in polar solvents. The resulting interactions, the electrokinetic potential measured as the zeta potential, will dictate both in vivo and in vitro particle behavior. Quality attributes thus include particle size distribution, pH, rate of settlement, viscosity, dissolution, zeta potential, and flocculation (formation of aggregates).

Within the framework of a suspension, there are several related dosage forms that differ from the perspective of its physical properties, impact on bioavailability, ease of use, and suitability for route of administration (e.g., oral, topical, parenteral). These include:

- Emulsion: Emulsions are a fluid system consisting of two immiscible phases, leading to liquid droplets (containing the API) which are dispersed in the solvent. The droplets may be amorphous, liquid–crystalline, or any mixture thereof. The diameters of the droplets constituting the dispersed phase usually range from approximately 10 to 100 μm (i.e., the droplets may exceed the usual size limits for colloidal particles). An emulsion is termed an oil/water (o/w) emulsion if the dispersed phase is an organic material and the continuous (solvent) phase is an aqueous solution. Conversely, a water/oil (w/o) emulsion consists of an aqueous dispersed phase that is dispersed in a hydrophobic (oil) phase (Desu et al., 2014). As is the case with other suspensions, emulsions are thermodynamically unstable, thereby necessitating the inclusion of an emulsifying agent to prevent the coalescence of the dispersed droplets (by a reduction in particle interfacial tension). Emulsions formulations typically include oil, preservative, antioxidants, emulsifying agents, buffers, and flavor. Challenges associated with the manufacture of emulsion dosage forms include flocculation or creaming, aggregation, phase inversion, and microbial contamination. Corresponding CQAs include viscosity, content, impurities, pH, rate of settlement, particle size, zeta potential, and aggregation (Nichols et al., 2002).

- Suspoemulsion: A suspoemulsion (SE) is a combination of a suspension and emulsion. It is a mixture of water-insoluble APIs dispersed in an aqueous solution, where one (or more) of the active ingredients is in suspension form and one (or more) of the active ingredients is in emulsion form. The formulation is intended for dilution into water prior to spray application. The continuous phase is comprised of water in which both solid particles and emulsion droplets distribute. This formulation type is especially suitable whenever there are two active substances with different solubility profiles. Suspoemulsions are easy to handle and measure, dust free, nonflammable, and offer good miscibility with water. However, they may need to be mixed before use because of potential stability problems.

Pastes: A semisolid dosage form containing a large proportion (20–50%) of solids finely dispersed in a fatty vehicle. Vehicles used in the formulation of paste and gels include aqueous solvents (least expensive and poses minimal toxicity), oil, or organic solvents. Challenges associated with the manufacture of pastes and gels include producing the desired viscosity, cohesiveness, lack of plasticity, and the need to avoid a separation of liquid and solid components of the formulation. Typically, glycerin, glycols, natural or synthetic gums, and polymers are used to increase the product viscosity while balancing formulation plasticity and cohesiveness. To overcome the separation of water from the gel, absorbing materials such as MCC, kaolin, colloidal silicon dioxide, and starch can be used. Preservatives may be added to inhibit microbial growth.

Gels: A semisolid system in which a liquid phase is constrained within a three-dimensional cross-linked matrix.

Lotions: Suspensions (emulsified liquid dosage form) intended for external application (e.g. calamine lotion, hydrocortisone lotion).

Cream: A cream is a semisolid emulsion systems (o/w, w/o) containing more than 10% of water. Oil in water creams are more common than water in oil because the former tends to be more comfortable to the user, due at least in part to the property of feeling less greasy.

Ointment: An ointment is a highly viscous or semisolid preparation intended for external application.

Types of Dosage Forms: Solids

Tablets: Tablets are compressed powders prepared by one of three general methods: wet granulation, dry granulation (roller compaction or slugging), and direct compression. Issues associated with manufacturing processes are described elsewhere is this chapter. Tablets can be classified on the basis of route of administration (e.g., oral, sublingual, rectal, vaginal, and subcutaneous implant) or formulation characteristics (e.g., immediate release, MR, effervescent, melt in mouth, chewable, flavored, or fast dissolving). In all cases, the general manufacturing processes, technique used for preparation of tablets, and materials are similar.

Immediate release (IR): IR tablets typically dissolve in gastrointestinal contents with no purposefully generated delay in drug dissolution or absorption.

Modified release (MR): MR tablets are manufactured with the purpose of altering the in vivo drug release/dissolution. MR tablets can be in the form of extended or delayed release.

Extended release (ER): Expressions such as "extended release", "prolonged-action," "repeat-action," and "sustained-release" have been used to describe these dosage forms. However, the term "extended-release" is used for pharmacopeial purposes.

ER tablets are formulated to cause the formulation rather than the API to serve as the rate-controlling factor in drug dissolution/release. In so doing, the API can be released over a prolonged duration of time. For orally administered products other than those intended for gastric retention, the success of the formulation is determined by its ability to be absorbed throughout the GI tract. Thus membrane permeability and GI transit time of the target animal species may limit the utility of these formulations in veterinary medicine.

ER formulations can be designed for first-order or zero-order drug release. First-order release is characterized by a specified percentage of the label dose being released over time. A zero-order release of drug is characterized by a specified amount of drug released over time. Chapter 2 should be consulted for more details on the impact of rate-limiting processes on drug pharmacokinetics. Preference is dictated by the absorption properties of the drug and the API exposure–response relationship. The altered drug release characteristics may be achieved through the use of matrix systems (e.g., hydrogels, polymeric matrix, and wax matrix), multiparticulate systems (e.g., granulates, pellets, and beads), polymeric film coating, ion exchange resins, pH-dependent release methods (e.g., enteric coating), and laser drilling. Some of these methods are best achieved by using a capsule rather than a tablet dosage form.

Delayed release: Generally, delayed release tablets are designed to constrain the release of the drug substance until the tablet has passed through the stomach. Delayed release tablets are desired when the drug may be destroyed or inactivated by the gastric juice or when it may irritate the gastric mucosa. Delayed release can be accomplished through the use of enteric coatings.

Bolus: The bolus is a very large tablet that is intended to be retained in the rumen. A slow dissolution process and ruminal retention provides an opportunity to achieve prolonged systemic drug exposure. The release of the active ingredient generally relies on erosion, diffusion from a reservoir, dissolution of a dispersed matrix, or an osmotic "driver." Regurgitation during rumination is prevented by formulating the bolus with a density of $\sim 3\ \text{g/cm}^3$. Critical quality attributes include assay, content uniformity, dissolution, hardness, friability, impurities, and loss on drying.

Implants: An implant is a small tablet that is manufactured in the same way as a tablet. It is a nonoral dosage form that can remain in the body of the animal for many months. They can be either immediate or MR. It is important to recognize that there are numerous variables that can influence the drug release properties of an implant. For example, drug can diffuse both vertically and horizontally. Therefore, the dimensions of the implant and the diffusivity of the drug within the implant matrix can have significant impact on the in vivo rate and extent of drug release. Furthermore, in some cases, implants are manufactured as multilayer products such that the diffusion of drug through each layer can be controlled. Lastly, there are also implants that work as hydrostatic pumps such that the drug is pushed out as water from the tissues move in (Kleiner et al., 2014). Critical quality attributes include assay, content uniformity, dissolution, impurities, and loss on drying.

Chewable tablets: The development of chewable tablets for dogs and cats started in the 1960s, a time when the companion animal pharmaceutical products were produced with the same or similar design and formulation characteristics as compared to the human pharmaceutical. In the mid-1970, the first "chewable" veterinary tablets were developed for dogs, manufactured using standard pharmaceutical processing equipment. Most of these chewable tablets were manufactured by wet granulation technology using water and corn syrup. The early canine chewable tablet formulations were associated with palatability scores of 70–85%. Feline free choice palatability did not fare as well, often with palatability scores of less than 50%. In the 1980s, milk and cheese flavors were tried in for both dogs and cats. Canine palatability never exceeded 80% free choice acceptance and feline palatability never exceeded 70%. Fruit flavors were common in companion animal chewable oral liquids, but fruits are not part of a companion animal's natural diet. Garlic, which has long been considered palatable to dogs, resulted in a free choice acceptance of only about 30–60%. When the garlic flavor was removed and a different flavor system used, a free choice level of 95% was achieved.

The initial palatability enhancing agents often included animal by-products of questionable quality and reproducibility. Common palatability enhancing agents included bovine pancreas digest, bovine liver extracts, bovine meat by-products, fish meal, fish digest, and other ingredients that were not fit for human consumption. The high fat content of these flavors made them prone to rancidity. Even if stabilized with antioxidants, rancidity was an important stability issue (Fahmy et al., 2008). Furthermore, the animal and fish by-products often had very high microbiological counts (greater than 50,000 cfu/g) and were contaminated with *Escherichia coli*, salmonella, and other coliform bacteria. This bacterial growth caused the chewable tablets to turn from brown to green and to emit offensive odors (Meijboon and Stronk, 1972).

In the early 1990s, the industry started to develop alternative chewable tablet formulations with improved flavoring agents that provided an attractive aroma and taste which led to an increase in free choice acceptance. Today, the new flavoring agents meet human food-grade and/or pharmaceutical grade quality standards, are stable and consistent, and contain negligible to nondetectable levels of bacteria, mold, yeast, and fungi.

Chewable tablets can be made by direct compression, wet granulation (using an appropriate solvent), or dry granulation (slugging or roller compaction), extrusion, or produced in a forming machine. "Direct compression" is easy to apply and necessitates minimal capital investment. As this new palatable chewable tablet is formulated, tablet weight and hardness become important variables. For example, when formulating feline chewable tablets, the generalized "ideal" hardness is in the range of 3–4 Kp. All other factors being held constant, as hardness exceeds 6 Kp, the palatability tends to decrease. The identical formulation at 6 Kp tablet hardness may have a 95% free choice acceptance in cats, but only 50% free choice when formulated with a 12 Kp tablet hardness.

The excipients are selected so that the blend can be formed into shapes through a forming machine. In this process, the dry components, which include the API, flavor, fillers, binder, lubricant, and any other ingredients, may be milled and blended. The solvents are added and are mixed until a dough is formed that has the desired texture. The dough batch is divided into portions in accordance with the sublot designation of the configuration being manufactured. The dough sublot is transferred to the forming machine hopper, producing a range of shapes or chews and dosage form weights that can be as high as 15 g. Once the chews are formed, they are taken

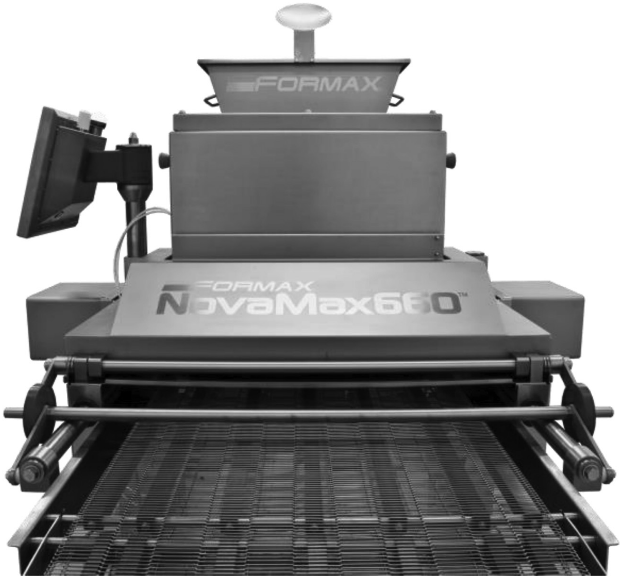

Figure 5.6 Forming machine for chewable dosage forms, *Source:* Courtesy of Provisur.

to either an oven at 40–50°C and/or left at room temperature with relative humidity 40–70% (for up to 7 days depend on the nature of the polymer or the formulation) for curing. An example of a chewable forming machine is provided in Figure 5.6.

Critical quality attributes include appearance, assay, content uniformity, dissolution, impurities, and loss on drying.

Capsules: Capsules are a solid dosage form in which the drug is enclosed in a hard or soft soluble shell. The shells are usually made from a gelatin, cellulose, or polymeric material. The shells may be "hard", consisting of two pieces (a body and a cap) that is typically filled with powder or beads. The hard capsules may be used as a mechanism for achieving extended drug release through such technologies as beads and osmotic pumps (Liu et al., 2014; Becker et al., 2014).

Alternatively, the shell may be "soft", consisting of a single piece (unit) that is typically filled with a solution or suspension containing the API. Soft capsules are often used for formulating poorly water-soluble drugs as a method of enhancing in vivo dissolution. Another advantage offered by the use of single unit capsules is that it avoids potential problems in achieving content uniformity.

The potential for undesirable formulation effects need also be considered when selecting between hard or soft shell capsule formulations. In particular, because the contact between the shell wall and its liquid contents is more intimate than in dry-filled capsules, there is an increased risk of such interactions as gelatin crosslinking and pellicle (thin skin or film) formation. Quality attributes of capsules include assay, content uniformity, impurities, and dissolution.

Powders: The API is mixed with other excipients to produce a final product for oral administration. Drug powders could be added in a solid form or feed, formulated as a soluble powder for addition to drinking water or milk replacer, or combined with emulsifying agents to facilitate their administration as liquid drenches. Challenges with powder dosage forms include undesirable taste attributes and potential problems with keeping denser powders from separating and concentrating at the bottom of the feed trough. Quality attributes include blend uniformity, impurities, and moisture.

Granules: This dosage form consists of powder particles that have been granulated with other excipients to form larger particles, usually 2–4 mm in diameter. Granulation enhances ease of blending (e.g., with other excipients) and blend uniformity.

Types of Dosage Forms: Medicated Feed

Type A medicated article consists of an API with or without a carrier (e.g., calcium carbonate, rice hull, corn, gluten) and with or without inactive ingredients. The Type A medicated article may be used to manufacture another Type A, or a medicated article that has been diluted with additional feed (Type B or a Type C). The Type A medicated article is manufactured by mixing or granulating excipients to the API. The API can be synthesized chemically or produced by a fermentation reaction. Adding excipients enhance the uniformity of the mix, prevent segregation of the drug, and prevent segregation during transportation. Excipients can also be added to prevent dust formation during the mixing and to stabilize the drug in medicated feed.

Types of Dosage Forms: Biomass Products

The FDA Guidance for Industry, *#216 Chemistry, Manufacturing, and Controls (CMC) Information—Fermentation-Derived Intermediates, Drug Substances, and Related Drug Products for Veterinary Medicinal Use* can be used as a general guide for the fermentation process used in biomass production.

Biomass APIs are an unpurified fermentation product. The contents of the fermentation vessel are dried to produce the biomass drug substance. Characterization of an API typically addresses the active molecule and impurities, which could include:

- the organism used in the fermentation, other metabolites produced by the organism, and components of the fermentation media;
- structural elucidation, which typically includes elemental analysis, mass spectrometry, infrared spectroscopy, ultraviolet spectroscopy, nuclear magnetic resonance spectroscopy, X-ray crystallography, and/or other appropriate tests. When the active ingredient is a mixture of related molecules, structural elucidation of isolated and purified individual components may be appropriate;
- physiochemical properties such as optical rotation, solubility profile, dissociation constant, and other properties that may affect performance of the finished product;
- biological activity, which includes drug potency and homogeneity;
- identity of the organism (genus, species, strain) and its source;
- identification of all metabolites with antimicrobial activity or toxicity. This may be determined through studies or scientific literature e.g., the "fingerprint" of the biomass.

Types of Dosage Forms: Controlled Internal Drug Release System

Controlled Internal Drug Release System (CIDR) is an intravaginal insert that is typically used in dairy cattle, goats, and sheep. The CIDR is a T-shaped device with flexible wings that collapse to form a rod which can be inserted into the vagina with an applicator. On the end opposite to the wings of the insert is a tail that is attached to facilitate removal of the device. The backbone of the CIDR is a nylon spine covered by a progesterone-impregnated silicone skin. The rate of progesterone release is influenced by the surface area of the implant and by the drug load. Upon its insertion, blood progesterone concentrations rise rapidly, with maximal concentrations reached within an hour. Progesterone systemic concentrations are maintained at a relatively constant level throughout the 7 days during which the insert is resident within the vagina. Upon its removal, systemic progesterone concentrations rapidly decline (Rathbone et al., 2002).

Types of Dosage Forms: Liquid or Semisolid Dosage Forms

Parenteral products: The formulation and manufacture of injectable dosage forms involves a different set of considerations than that associated with oral products. The need for microbiological stability is the primary difference. Vials of injectable formulations may be concluded to be sterile upon release for marketing, but once the stopper is punctured, sterility can no longer be assured. Multiuse injection products must therefore be formulated with sufficient concentrations of bacteriostatic/fungistatic agents such as methylparaben to avoid growth of microorganisms in the remaining vial contents.

As with solid pharmaceuticals, injections may be formulated as either IR or MR dosage forms. Since the in vivo residence time of injectable formulations are not constrained by GI transit times, there are few limitations on the duration of drug release from these dosage forms. To achieve the desired time release characteristics, formulators can manipulate product physical properties such as viscosity and particle size.

One of the critical considerations when manufacturing parenteral dosage forms is the need to insure product sterility. Selection of an appropriate process necessitates an understanding of the potential chemical reactions that can occur. Accordingly, the strengths and limitations of the various sterilization processes depend on physical aspects unique to the type of pharmaceutical. In this regard, it is important to note that sterilization drives design of manufacturing processes, facilities, and formulation of sterile injections.

Common sterilization techniques used for parenteral products include:

- Sterile filtration/aseptic processing: sterilization is achieved by passing the solution through a filter designed to retain bacteria.
- Terminal sterilization: product sterilization after the product is already packaged. Types of terminal sterilization processes include:
  - Moist heat terminal sterilization: Terminal sterilization by the use of an autoclave. Terminal moist heat sterilization is often preferred for aqueous solutions.
  - Radiation terminal sterilization: Products are conveyed to an ionizing radiation source, ensuring that each unit receives the amount of radiation exposure necessary to kill the microbes within the unit. Gamma irradiation, for example, kills bacteria by inducing the formation of free radicals that damage nucleic acids within the microorganisms (Savjani et al., 2012). This method may be more appropriate for heat-labile materials.

Common types of injectable formulations include:

- injectable aqueous solutions;
- injectable aqueous suspensions;
- injectable organic solutions/suspensions;
- lyophilized (freeze-dried) powders for injection.

Analytical testing of injection dosage forms may include the following parameters.

- Appearance/description: In addition to the general color, opacity, and appearance of the product, the existence of *visible* particulate matter should be noted. Subvisible particulates are enumerated in the Particulate Matter Test, described below.
- Identification
- pH
- Viscosity (if appropriate)
- Assay for active ingredient
- Assay for preservatives (if appropriate): For multiuse products, a liquid or gas chromatography method may be used to detect levels of antimicrobial preservatives. The manufacturer must demonstrate that the limits for concentrations of preservatives still represent levels that effectively inhibit microbial growth. This test is omitted if the product is single-use only.
- Water: A water test is generally only appropriate for lyophilized powder or nonaqueous injections.
- Impurities
- Dissolution/drug release (if appropriate): If an injection is considered immediate release, this test is typically omitted from the testing battery. For MR injections, a manufacturer may develop a test similar to those used for oral dosage forms according to USP guidelines, but this is not practical in cases where the release from the product in vitro extends more than a few days. Often, in vitro dissolution or drug release tests must be "accelerated" by the use of organic solvents or by the use of dissolution media that allow for the completion of the test in a reasonable period of time.
- Particulate matter (IV use): This test measures the number of subvisible particles, specifically those 25 μm in diameter or less, and is different from the Appearance/ Description visible check for particles, described above. These tests are performed using light obscuration (laser scattering) or microscopy. Veterinary products that are not intended for IV use are exempt from this testing.
- Bacterial endotoxins: Gram-negative bacteria present during the manufacturing process release lipopolysaccharides, known as endotoxins, upon the death of the bacteria (Booth, 2001). Endotoxins are antigenic to most target species and cannot be completely eliminated by the most common sterilization techniques. USP Chapter <85> (USP, 2009b) describes tests to measure endotoxin levels (with results expressed either as Endotoxin Units (EU) or as International Units (IU)). Endotoxin limits for products administered by IM, SC, or IV routes historically have been based on the rabbit endotoxin sensitivity of about 5 EU/kg body weight divided by the dose of the injection per kilogram body weight (Rahman et al., 2014). Although it is recognized that each target animal may differ in its sensitivity to endotoxins, unique endotoxin limits have not been developed for individual species.
- Sterility: Tests for sterility usually follow one of the procedures described in USP Chapter <71> (USP, 2009a). In general, it is a requirement that all injection dosage forms be free of microbial contamination (i.e. sterile) before being marketed.

Types of Dosage Forms: Topical Dosage Forms

Topical products may be formulated as solutions, suspensions, ointments, gels, creams, emulsions, or aerosol. Unlike transdermal formulations, these products are not intended to be systemically absorbed. The location of delivery can be the skin, the mammary gland, the eyes, or the ears. The location of the application dictates the appropriate excipients to insure that the API reaches and is retained within the site of action. One of the challenges encountered with these products is that systemic drug concentrations (e.g., blood level profiles) cannot be used to evaluate product performance. As a result, the formulation chemist needs to rely upon in vitro and physicochemical tests to evaluate the impact of formulation and manufacturing method on product CQAs. In turn, without extensive clinical trials and/or in situ model studies, allowable deviations in product physicochemical characteristics will be very difficult to define (Teng et al., 2009).

Types of Dosage Forms: Transdermal Drug Delivery

Formulating the API so that it penetrates protective barriers such as skin can be a significant challenge. A number of complex systems have been developed to accomplish this goal including patches, ointments, suspensions, lotions, plasters, pastes, and dressings. Within this textbook, Chapters 2 and 47 discuss this topic further.

Manufacturing Process

The manufacturing process can have as great an influence on drug product quality and performance as the formulation. For that reason, the manufacturing process needs to be considered both from the perspective of the dosage form, batch size, and physicochemical properties of the drug and its excipients. As mentioned under our discussion of lubricants, something as simple as blending time or the order of excipient addition can make the difference between products that exhibit the desired in vivo characteristics versus those failing to exhibit the targeted quality attributes.

Wet/dry granulation: The tablet manufacturing process can be classified as granulation (wet or dry granulation) or direct compression. Granulation is a process that causes an increase in particle size, thereby improving the flow, density, and compressibility of the powder (API and excipients). Granulation can be achieved by using a binder solution (wet granulation) or a dry granulation (without the addition of a binder solution).

Wet granulation is the most commonly used method to manufacture veterinary tablets. Wet granulation (low shear, high shear, fluid bed, extrusion, and spheronization process of converting material into spheres) is a process of using a liquid binder (usually a hydrophilic colloid) to lightly agglomerate the powder mixture. This reduces the interparticulate friction and improves the fluidity and compressibility of the powder. The binder, which is distributed over large surface areas, acts as glue to overcome the lack of cohesiveness of the original API and the fillers. Wet granulation also improves the blend uniformity for soluble low-dosage drugs and is an effective technique to improve the dissolution rate of hydrophobic compounds. The quality of this technique is generally governed by the physicochemical properties of the powder and/or granulation from which tablets are composed. Wet granulation may not be suitable for an API that is sensitive to humidity or temperature.

Dry granulation (roller compaction or slugging) is a process that creates granules by compaction of the powder blend under low pressures. This process often results in ribbons that are subsequently milled and screened to form granules of the desired particle size. The advantage of dry granulation is the absence of heat and moisture. However, it can lead to dust formation and may not be as efficient as wet granulation in terms of insuring the uniformity of the API content in the resulting granules.

Direct compression: Direct compression is the tableting of ingredients in the absence of a preliminary granulation step. This process avoids many of the problems associated with wet and dry granulation and is best used for formulations that exhibit good flowability, compactibility, and compressibility. However, the product generated by direction compression methods is highly dependent on the intrinsic physical properties of the raw materials (especially fillers). Any variability in the quality of the raw materials may affect the flow and compression characteristics, causing the direct compression to be challenging. In an effort to overcome this hurdle, some excipients are now available to facilitate direct compression (e.g., fast flow lactose, dicalcium phosphate dibasic, microcrystalline cellulose). These excipients are manufactured consistently to assure their physical forms (e.g., particle size, particle shape), thereby facilitating improved formulation flowability and compressibility.

Extrusion: Extrusion processes can be categorized as either ram extrusion or screw extrusion. Either technique is well suited for the precision extrusion of highly valuable materials. Process control monitoring includes zone temperature, the monitoring of torque, drive amperage, and pressure and melt viscosity. Temperatures are normally controlled by electrical heating bands and monitored by thermocouples.

Roller compaction: Recently, dry granulation using roller compaction has grown in popularity, as the process is economical, energy efficient, easily automated, and suitable for drugs that are sensitive to heat and moisture (Kona et al., 2014). In roller compaction, the powder blend is compressed and compacted between two counter-rotating rollers resulting in ribbons, which when milled produce granules of the desired size (Kostewicz et al., 2014). Following the use of roller compaction, the resulting granules are mixed with lubricant and/or other excipients and finally compressed into the tablets.

Slugging: Slugging is used to compress the formulation into relatively large tablets that are subsequently milled and screened to attain the desired size granules. The resulting granules are blended with lubricant and/or any other excipients and then finally compressed into tablets.

Tableting processes: The size and speed of tableting machines (presses) can vary, but their general operating principles are the same. Tableting processes typically use a powder blend or granulation. The powder blend or granulation is added to a hopper, which then flows in a controlled manner into dies. Above and below each die are punches that create the compression pressure necessary to form the tablet into the appropriate shape. Tablet shape is determined by the geometry of the die. Following compression, the newly formed tablets are ejected from the press and the process repeats.

Factors Influencing Product Dissolution

Upon examination of the attributes of the dosage forms described in this chapter, it is evident that the ability of the drug to dissolve in and diffuse through biological fluids is an essential component of product performance. The fundamental issue is developing an understanding of the physicochemical properties of the API and how to adjust the formulation so as to accommodate the critical factors influencing in vivo product performance. With respect to product dissolution, the critical factors can be identified in the Noyes–Whitney equation (Equation 5.9).

$$\frac{dC}{dt} = K \times S\,(Cs - C) \tag{5.9}$$

Where

dc is the change in concentration of drug in solution
C is the concentration of drug in solution
dt is the change in time (expressed in the units of interest)

Cs is the solubility of the drug in the media
S is the surface area of the solid drug
K is a constant describing the diffusion of the drug in the media

This equation can be further modified to include terms pertaining to diffusion layer thickness, particle density, and particle shape. These additional terms have been incorporated into in silico physiologically based pharmacokinetic models to predict in vivo drug dissolution (Lennernäs et al., 2014; Brouwers et al., 2009).

Some techniques to increase product dissolution rate include reducing the particle size of the drug to increase the surface area, adding excipients to form micelles and enhance solubility in the diffusion layer thickness, or use of a highly water-soluble salt of the parent drug substance. Changes in saturation solubility are now being incorporated as mechanisms for achieving "supersaturation" whereby drugs that would typically precipitate in the gastrointestinal tract now remain in solution for a longer duration, thereby facilitating drug absorption (Waterman et al., 2006). By combining information on the drug absorption capability of the various intestinal segments, drug physicochemical properties (including pKa and aqueous solubility) and formulation dissolution rate, we can predict the likely in vivo blood level profiles.

Putting it All Together: Product Quality Control

Efforts of the pharmaceutical scientist culminates in the manufacture of a product that provides the desired in vivo and in vitro release characteristics. Once a formulation and the corresponding manufacturing method is finalized and that product is determined to exhibit the desired clinical and physicochemical attributes, there is the need to insure that all future batches will perform in a likewise manner. Quality cannot be tested into a product; rather, it is the culmination of product/process understanding, in-process controls and adhering to cGMPs that provide a greater assurance of drug quality. Many of the parameters that we discuss here become translated into batch release specifications (i.e., test results that must be met in order to release the batch or lot for public distribution).

Conclusion

The development of a pharmaceutical formulation that succeeds in meeting the desired rate and extent of in vivo drug exposure is possible only through an understanding of the physicochemical attributes of the API. In turn, the appropriate dosage form and excipients need to be defined. However, as we have emphasized, a drug recipe is certainly not sufficient to insure achieving the desired drug product performance. The order in which ingredients are added, the kinds of equipment used, the quality of the excipients, and the ability to understand and achieve tight control over the manufacturing process are no less important than the formulation. Appreciating these nuances in the principles of pharmaceutics lends not only to the development of high-quality safe and effective dosage forms but also allows for an assurance of each production batch meeting consumer expectation and an understanding of the veterinarian as to challenges that may be encountered when a specific product is administered to the animal patient.

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Section 2

Drugs Acting on the Autonomic Nervous System

6

Introduction to the Autonomic Nervous System and Autonomic Pharmacology

Joshua A. Rowe, Rose M. McMurphy, Barbara J. Lutjemeier, and Michael J. Kenney

Introduction

The autonomic nervous system (ANS) includes afferent, central, and efferent components. The efferent component is the general visceral efferent (GVE) system and is subdivided into two primary branches, the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS). More recently, the enteric nervous system is considered a functional component. The ANS plays a critical role in regulating processes required for maintaining physiological homeostasis and responding to acute stressors. Numerous physiological functions are regulated by the ANS including, but not limited to: regulation of heart rate and cardiac contractility; visceral and cutaneous blood flow distribution; gastrointestinal motility and digestion; and urogenital processes. It is virtually impossible to consider physiological regulation without integrating the roles of sympathetic and parasympathetic neural mechanisms into a functional overview, as summarized in the *Primer on the Autonomic Nervous System* (Hamill and Shapiro, 2004):

> The autonomic nervous system (ANS) is structurally and functionally positioned to interface between the internal and external milieu, coordinating bodily functions to ensure homeostasis (cardiovascular and respiratory control, thermal regulation, gastrointestinal motility, urinary and bowel excretory functions, reproduction, and metabolic and endocrine physiology), and adaptive responses to stress (flight or fight response). Thus, the ANS has the daunting task of ensuring the survival and the procreation of the species.

For the most part, physiological processes regulated by the ANS are not under voluntary control and are essential for maintaining physiological regulation under basal or resting conditions, as well as in response to various forms of physical or emotional stress. Changes in the level of afferent and efferent activity in sympathetic and parasympathetic nerves occur primarily independent of conscious or voluntary control, thereby providing the derivation for the name autonomic nervous system (from the Greek: *auto* = self; *nomos* = law).

Sympathetic Nervous System

Sympathetic nerves originate from cell bodies in the intermediolateral cell column of the thoracic (T) and lumbar (L) sections of the spinal cord, synapse in ganglia (more anatomical detail is presented later in this chapter), and project to a wide array of targets (Figure 6.1). The level of activity in sympathetic nerves is regulated at multiple levels of the brain, including hypothalamic and brainstem sympathetic neural circuits (Figures 6.1 and 6.2). The majority of sympathetic nerves demonstrate a tonic or basal level of activity, characterized by the presence of bursts of activity (Claassen et al., 1996). Figure 6.3A shows original traces of directly recorded nerve discharge bursts from four sympathetic nerves (renal, adrenal, splanchnic, lumbar). Direct recordings of sympathetic nerve activity provide an output measure of central sympathetic neural circuits. Changing the level of activity in peripheral sympathetic nerves, by either increasing or decreasing the level of sympathetic nerve discharge (SND), is a primary means by which the SNS regulates physiological function (Kenney, 2014; Kenney et al., 2014). For example, heat stress or hyperthermia increases the level of visceral SND (Figure 6.3B), thereby mediating a redistribution of blood flow away from visceral organs to cutaneous vascular beds. On the other hand, acute increases in arterial blood pressure activate the arterial baroreceptor reflex which abruptly reduces SND (Figure 6.3C), a physiological response that contributes to returning blood pressure to normal levels. In this example (Figure 6.3C) blood pressure was increased by the intravenous administration of the adrenergic agonist drug, phenylephrine hydrochloride, which will be discussed in more detail in Chapter 7.

In many contexts the primary understanding of SNS regulation has focused on the activation of this nervous system as a unit, as in response to critical emergencies

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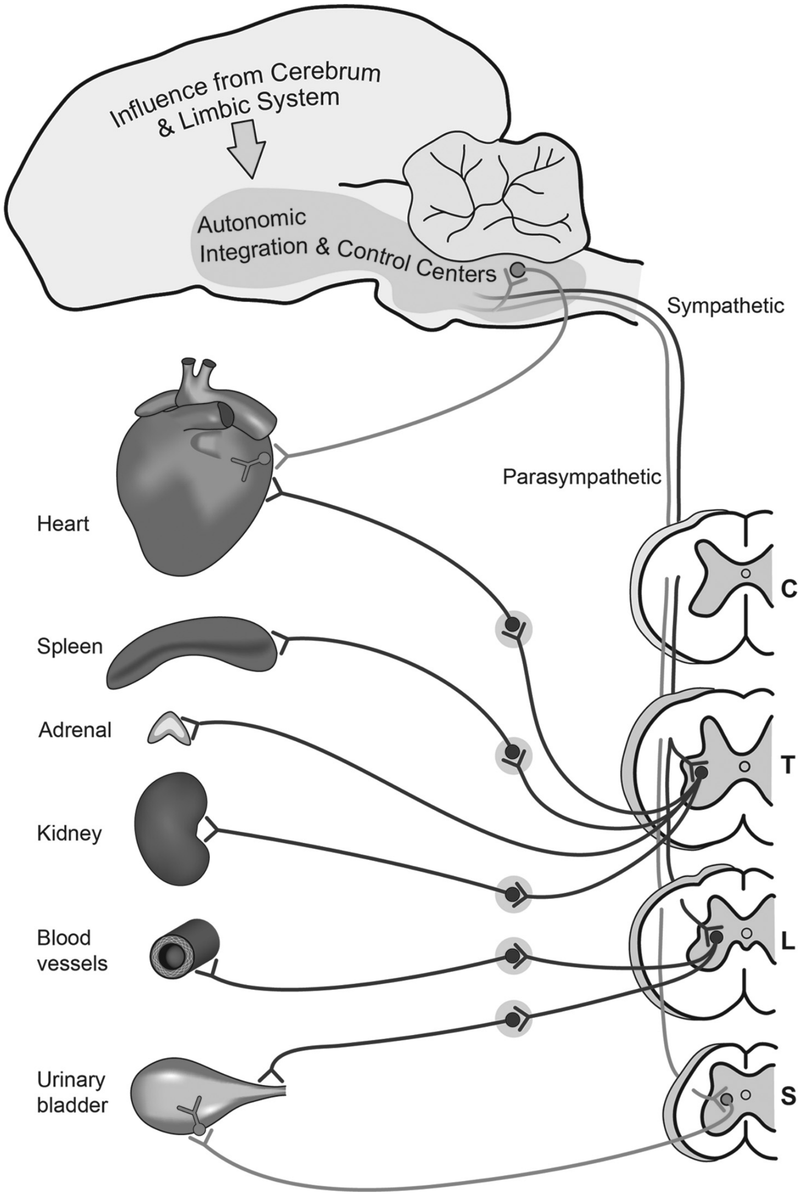

Figure 6.1 Schematic diagram highlighting anatomical features of the autonomic nervous system. Sympathetic preganglionic neurons originate from cell bodies located in the intermediolateral cell column of the thoracic (T) and lumbar (L) sections of the spinal cord, synapse in ganglia located outside the spinal cord, and postganglionic neurons project from the sympathetic ganglia to a wide array of targets. The chromaffin cells of the adrenal medulla are analogous to ganglionic neurons and are innervated by sympathetic preganglionic neurons. Efferent activity in sympathetic nerves is regulated by various integration and control centers located at multiple levels of the supraspinal neuraxis (identified as Autonomic Integration and Control Centers). Presympathetic neurons from these hypothalamic and brainstem sites project to the thoracic and lumbar spinal cord sites where the sympathetic preganglionic cell bodies are located. Parasympathetic preganglionic neurons originate from cell bodies located in brainstem nuclei and in the intermediolateral cell column of the sacral (S) spinal cord. Parasympathetic preganglionic neurons are innervated by nerve endings of preparasympathetic neurons whose cell bodies are found in supraspinal nuclei. Many parasympathetic preganglionic neurons terminate and synapse in intramural ganglia located within the innervated target organs, or in ganglia located outside but near the innervated target organs. C, cervical spinal cord.

or to elicit the “fight-or-flight” response. In these conditions, SNS activation mediates increased heart rate and cardiac contractility, vascular vasoconstriction in skin and viscera with shunting of blood flow to skeletal muscles, hepatic glycogenolysis, bronchiolar and pupillary dilation, and contraction of the spleen. This physiological response profile is mediated by the combined activation of peripheral sympathetic nerves innervating specific target organs (e.g., heart, blood vessels) and the adrenal medulla. This prominent activation state led to the concept that the SNS acts as a unit. However, it is now well-understood that a fundamental regulatory strategy of the SNS involves selectively controlling the level of activity in nerves innervating different targets in response to a number of physiological conditions (i.e., nonuniform regulation of sympathetic nerve activity). For example, acute cold stress or hypothermia increases the level of activity in nerves innervating cutaneous blood vessels but reduces the level of activity in nerves innervating the kidney, providing the neural substrate for reducing blood flow to the periphery and increasing visceral organ blood flow. The ability to selectively regulate the level of activity in sympathetic nerves innervating specific targets provides the neural substrate for producing highly specific physiological response profiles. Physiological responses of selected organs and effector tissues elicited by activation of efferent sympathetic nerves are summarized in Table 6.1. Specific receptor classes and types involved in mediating SNS-induced physiological responses are included in Table 6.1 and are introduced

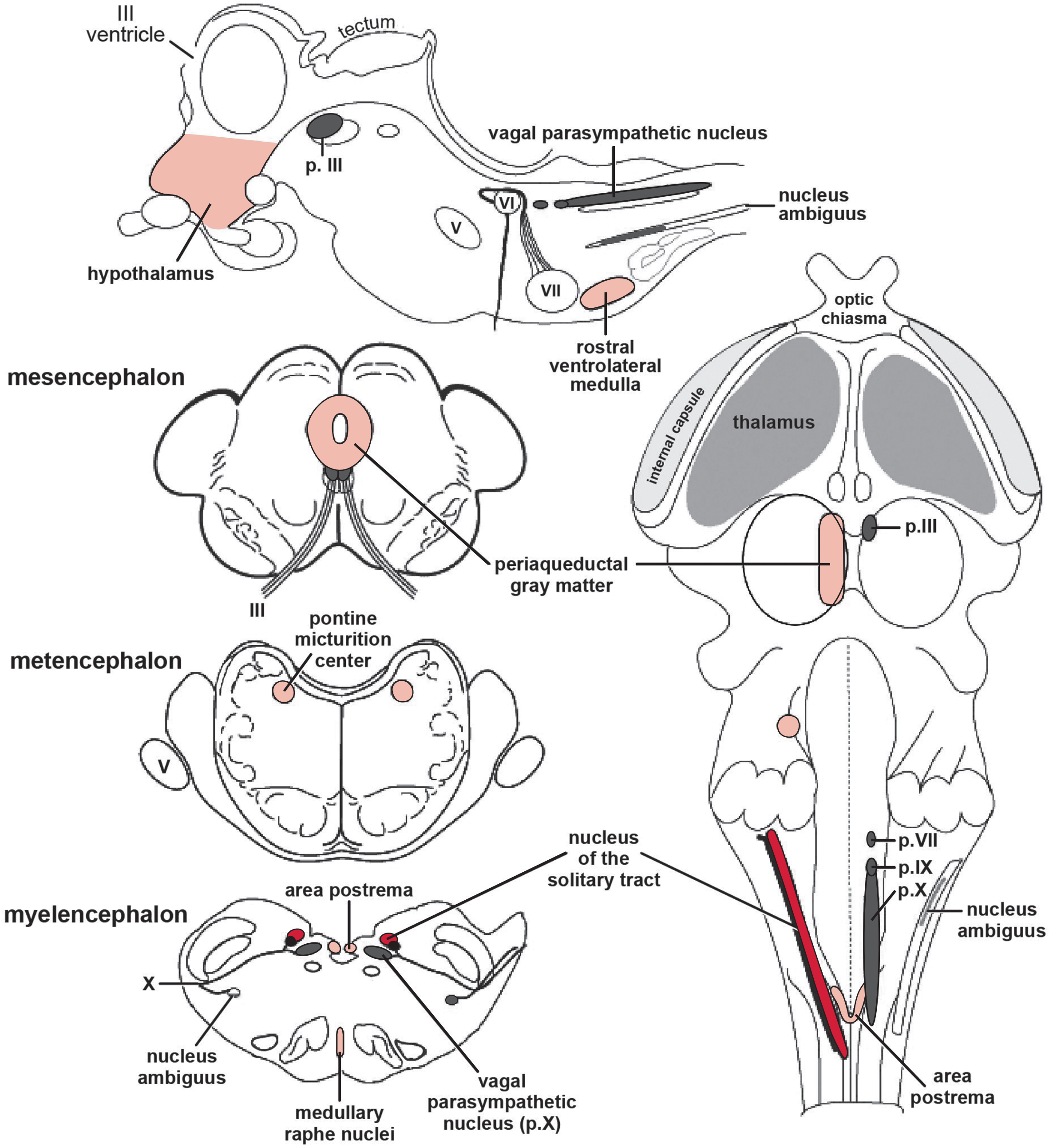

Figure 6.2 Selected autonomic nuclei and centers are shown in transverse (left), dorsal (right), and midsagittal (top) views. Components depicted in light red represent autonomic integration, relay, and/or control centers. The nucleus of the solitary tract, an important visceral sensory nucleus, is red. Brainstem preganglionic parasympathetic nuclei (p.) are shown in dark gray (including a portion of the nucleus ambiguous which provides cardiac visceral efferent neurons). Bilateral structures are shown on one side only in the dorsal view and not all structures are represented in each view to minimize clutter. The hypothalamus is the principle integration and control center of the autonomic nervous system and contains numerous nuclei, which are involved in the regulation of autonomic function. The periaqueductal gray matter surrounds the mesencephalic aqueduct and serves (among other functions) as a relay for visceral control signals from the hypothalamus to hindbrain nuclei. The pontine micturition center (just one of several autonomic control centers in the brainstem which control visceral functions) contains neurons that project to the lumbosacral spinal cord where they excite sacral preganglionic parasympathetic neurons which cause contraction of the urinary bladder wall and inhibit neurons to urinary sphincters (lumbar preganglionic sympathetic and sacral somatic neurons). The rostral ventrolateral medulla and medullary raphe nuclei are major sites of presympathetic neurons that project to preganglionic sympathetic neurons in the thoracolumbar spinal cord. The area postrema (just one of several circumventricular organs which are located in close proximity to the ventricular system of the brain and lack a blood–brain barrier) detects emetic agents in the blood and projects to hindbrain nuclei responsible for controlling emesis; other circumventricular organs (not shown) are likewise susceptible to direct chemosensory stimulation by blood-borne agents and are involved in other aspects of neuroendocrine and autonomic function. Source: Adapted from Fletcher and Brown, 2010.

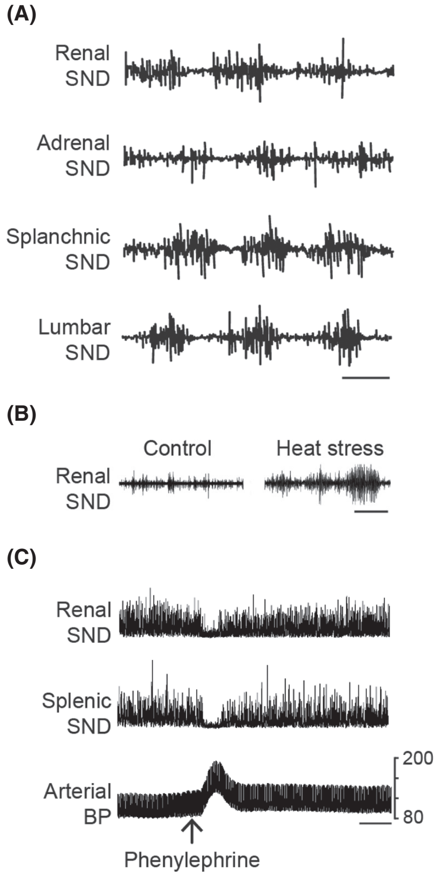

Figure 6.3 **(A)** Traces of sympathetic nerve discharge (SND) recorded under basal conditions from renal, adrenal, splanchnic, and lumbar nerves. Note that at rest sympathetic nerves are characterized by the presence of a tonic level of activity. Horizontal calibration is 100 ms. Source: Adapted from Claassen et al., 1996. **(B)** Traces of renal SND recorded under control conditions and during heat stress. Renal SND was increased from control levels during the period of acute heat stress. Horizontal calibration is 250 ms. Source: Adapted from Kenney, 2014. Reproduced with permission of Elsevier. **(C)** Traces of discharges from renal and splenic sympathetic nerves recorded before, during, and after an acute increase in arterial blood pressure produce by the intravenous administration of the α-adrenergic receptor agonist, phenylephrine hydrochloride. The acute increase in arterial blood pressure and subsequent activation of the arterial baroreceptors elicited a reflex-mediated inhibition of SND. Horizontal calibration is 15 s. Source: Adapted from Kenney, 2014. Reproduced with permission of Elsevier

later in this chapter and considered in more detail in Chapter 7.

Parasympathetic Nervous System

Parasympathetic nerves originate from cell bodies in the brainstem and sacral sections of the spinal cord, and synapse in ganglia (more anatomical detail is presented later in this chapter). The level of activity in parasympathetic nerves is regulated by multiple areas in the brain (Figures 6.1 and 6.2). Parasympathetic nerves innervating many target organs are tonically active and changing the level of activity in peripheral parasympathetic nerves in response to specific physiological stimuli is a primary mechanism by which the PSNS regulates physiological function. Figure 6.4 shows directly recorded cardiac vagal parasympathetic nerve discharge under basal conditions, and in response to an experimentally induced activation of the baroreceptor reflex, which produced an increase in the level of cardiac vagal nerve activity and an associated reduction in heart rate (Simms et al., 2007). The primary functional profile elicited by activation of the PSNS includes initiating and sustaining energy conservation and homeostasis during periods of relative physiological quiescence. In general, increasing the level of activity in parasympathetic nerves reduces heart rate, stimulates gastrointestinal secretions and peristalsis, contracts the body of the urinary bladder, and modulates immune function. Physiological responses of selected organs and effector tissues elicited by activation of efferent parasympathetic nerves are summarized in Table 6.1. Specific receptor classes and types involved in mediating PSNS-induced physiological responses are included in Table 6.1, and are introduced later in this chapter and considered in more detail in Chapter 7.

Many organs and tissues are innervated by both the sympathetic and parasympathetic arms of the ANS, and these targets are described as receiving dual ANS innervation (e.g., heart and urinary bladder in Figure 6.1). In many instances physiological responses produced by activation of parasympathetic or sympathetic nerves to a target tissue or organ that receives dual ANS innervation are functionally antagonistic. That is, if activation of one arm of the ANS inhibits a specific physiological function then activation of the other arm enhances the function. For example, stimulation of cardiac sympathetic nerves increases heart rate whereas activation of cardiac parasympathetic nerves reduces heart rate. However, the presence of a dual ANS innervation to a specific target does not indicate that the physiological function of that target is balanced to the same degree for each arm of the ANS. On the contrary, the basal physiological function of many organs is weighted towards either PSNS or SNS regulation, an effect that is species dependent and can vary depending on the specific physiological situation or pathophysiological state (Box 6.1 Case study). For example, the gastrointestinal system is regulated by the enteric nervous system, along with the SNS and the PSNS. Activation of parasympathetic nerves innervating the gastrointestinal tract and administration of drugs that mimic the PSNS stimulate gastrointestinal functions, whereas it is generally considered that the SNS exerts more of a modulatory effect on gastrointestinal function consistent with the fact that sympathomimetic drugs are not prominently used as GI inhibitory agents

Table 6.1 Physiological responses of selected organs and effector tissues produced by activation of efferent sympathetic (sympathetic stimulation) and parasympathetic (parasympathetic stimulation) nerves. Specific receptor classes and types involved in mediating the physiological responses are included for sympathetic (adrenergic receptors; α and β) and parasympathetic (muscarinic receptors; M) stimulation. Stimulation of sympathetic nerves innervating the adrenal medulla mediates secretion of epinephrine (EPI), an effect involving activation of nicotinic neural (N_N) receptors

Organs/effector tissues	Sympathetic stimulation	Receptor	Parasympathetic stimulation	Receptor
Eye				
Radial muscle, iris	Pupillary dilation	α_1		
Sphincter muscle, iris			Pupillary constriction	M_3, M_2
Ciliary muscle	Slight relaxation	β_2	Contraction	M_3, M_2
Glands of head				
Lacrimal	↑ Secretion (minor effect)	α_1	↑↑ Secretion (major effect)	M_3, M_2
Salivary	↑ Secretion (minor effect)	α_1	↑↑ Secretion (major effect)	M_3, M_2
Lungs				
Bronchiolar smooth muscle	Bronchiolar dilation	β_2	Contraction	$M_2 = M_3$
Heart				
Sinoatrial node	↑ Heart rate	$\beta_1 > \beta_2$	↓ Heart rate	$M_2 >> M_3$
Atria	↑ Contractility/conduction	$\beta_1 > \beta_2$	↓ Contractility/conduction	$M_2 >> M_3$
Atrioventricular node	↑ Automaticity/conduction	$\beta_1 > \beta_2$	↓ Conduction	$M_2 >> M_3$
Ventricle	↑ Contractility/conduction	$\beta_1 > \beta_2$	↓ Contractility	$M_2 >> M_3$
Blood vessels (arteries and arterioles)				
Coronary	Constriction; dilation	α_1, α_2; β_2		
Pulmonary	Constriction; dilation	α_1; β_2		
Skin and mucosa	Constriction	α_1, α_2		
Skeletal muscle	Constriction; dilation	α_1; β_2	Dilation (not due to parasympathetic stimulation)	M_2
Abdominal viscera	Constriction; dilation	α_1; β_2		
Gastrointestinal tract				
Motility	↓ Motility	α_1, α_2, β_1, β_2	↑ Motility	$M_2 = M_3$
Sphincters	↑ Tone	α_1	↓ Tone	M_3, M_2
Secretion	Inhibition	α_2	Stimulation	M_3, M_2
Urinary bladder				
Detrusor muscle	Relaxation	β_2	Contraction	$M_3 > M_2$
Sphincters	Contraction	α_1	Relaxation	$M_3 > M_2$
Sex organs				
Male	Ejaculation	α_1	Erection	M_3
Female			Erection	
Skin				
Sweat glands		α_1		M_3, M_2
Pilomotor muscles		α_1		
Adrenal medulla	Secretion of EPI	N_N		
Kidney				
Renin	↑ Secretion	β_1		
Liver	Glycogenolysis	α_1		
	Gluconeogenesis	β_2		
Splenic capsule	Contraction	α_1		
Autonomic nerves				
Sympathetic nerves				
Autoreceptor	Inhibition of NE release	α_2		
Heteroreceptor			Inhibition of NE release	M_2, M_4
Parasympathetic nerves				
Autoreceptor			Inhibition of ACh release	M_2, M_4
Heteroreceptor	Inhibition ACh release	α_2		

in clinical conditions. Therefore, the gastrointestinal system receives a dual ANS innervation and the physiological responses mediated by these nervous systems are functionally antagonistic. However, the fundamental physiological regulatory processes are weighted towards PSNS dominance. Moreover, in some cases the dual SNS and PNSN innervation to a specific tissue may produce similar yet not identical responses. For example, the salivary glands receive a dual ANS innervation, although activation of the SNS and PSNS do not produce

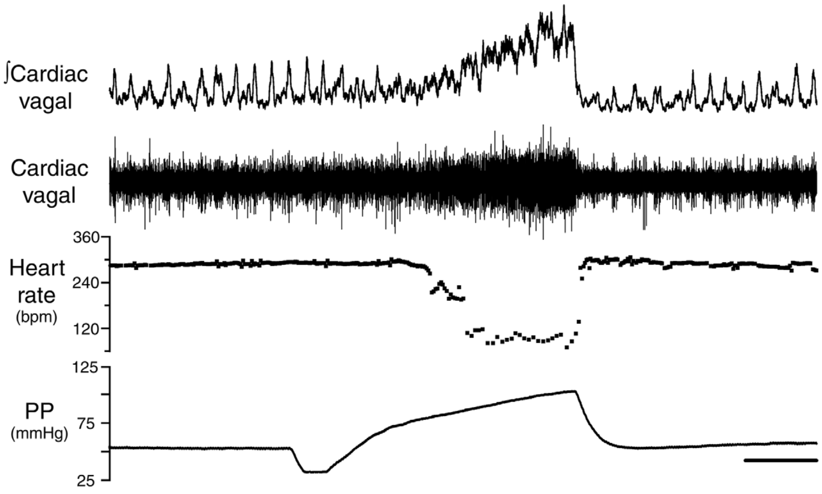

Figure 6.4 Traces of cardiac vagal nerve activity and heart rate recorded before, during, and after activation of the baroreflex produced by experimental manipulation of aortic perfusion pressure (PP). Note the presence of a tonic level of cardiac vagal nerve activity, the increase in vagal nerve activity in response to baroreflex activation, and the expected reflex-mediated reduction in heart rate in response to the increase in cardiac vagal nerve activity. Source: Adapted from Simms, 2007. Reproduced with permission of John Wiley & Sons.

functionally antagonistic responses. Activation of the SNS innervation to the salivary glands produces secretion of amylase and a viscous salivary fluid (secondary to activation of α-adrenergic receptors) whereas activation of PSNS innervation to the salivary glands produces a watery salivary fluid.

The ANS also contains extensive afferent (sensory) components that provide neural information regarding the internal physiological environment. The peripheral afferent components, namely the general visceral afferent (GVA) system, generally utilize similar nerve pathways as the efferent components. For example, the vagus nerve and its branches are comprised of approximately 80% afferent fibers (sensory) and 20% efferent fibers (DuBois and Foley, 1936). Afferent pathways conveying peripheral sensory information ultimately project to multiple supraspinal sites, including the hypothalamus and brainstem (e.g., the nucleus of the solitary tract). Integration of neural information at central sites can modulate the level of efferent activity in sympathetic and parasympathetic nerves.

It has often been considered that the ANS operates rather independently of other adaptive systems. However, the functional repertoire of the ANS now includes an important role for both arms of this nervous system in regulating and integrating processes between

Box 6.1 Case Study

An 8-year-old, female, spayed mixed breed dog was presented for acute onset of vomiting and lethargy. Laboratory data were consistent with mild dehydration. Vomiting continued despite treatment with intravenous fluid and antibiotics over the next 24 hours for presumed gastritis. Abdominal radiographs and ultrasound were negative for gastrointestinal foreign body or obstruction. No peristalsis was evident on ultrasound. The vomiting subsided but the patient became progressively more lethargic and unable to ambulate more than a few feet. The patient refused to eat. Fecal incontinence was present secondary to decreased anal sphincter tone. Although the dog was initially able to urinate, the volume of urine she was able to expel progressively decreased over several days until she was unable to void. Photophobia developed and the pupillary light reflex became sluggish. An echocardiogram revealed an ejection fraction of only 14%. The heart rate remained near 85 beats per minute despite this low ejection fraction. A diagnosis of dysautonomia was made (Harkin et al., 2002; Harkin et al., 2009).

Dysautonomia is an idiopathic neurodegenerative disorder characterized by degeneration of neurons in ANS ganglia, which produces marked changes in the function of target organs innervated by the sympathetic and parasympathetic nervous systems (Harkin et al., 2002; Harkin et al., 2009). Due to the widespread innervation of the ANS to numerous tissues and organs, patients with dysautonomia present with alterations in multiple physiological systems, making this disease state difficult to diagnose. Changes in target function are generally manifested based on the usual predominance of sympathetic or parasympathetic tone to a given organ or tissue. For example, under basal conditions, function of the gastrointestinal tract is dominated by a predominance of parasympathetic nerve activity conveyed via the vagus nerve and manifested as enhanced gastrointestinal motility and activity. Therefore, ganglionic degeneration and loss of the PSNS innervation to the gastrointestinal tract is often characterized by reduced smooth muscle tone and motility in patients suffering from dysautonomia.

diverse physiological systems. For example, it is well-established that the ANS plays a role in mediating interactions between the nervous and immune systems, two adaptive systems that have generally been considered to function independently of each other (Kenney and Ganta, 2014).

Multiple experimental methods have been employed for assessing SNS and PSNS contributions to physiological states and pathophysiological conditions including: pharmacological activation or blockade of receptors associated with the sympathetic and parasympathetic nervous systems; molecular approaches involving genomic and proteomic analyses; direct recordings of afferent and efferent activity in sympathetic and parasympathetic nerves; and the use of multiple methods to analyze specific components of directly recorded nerve activity. The collective contributions of these studies have provided essential information regarding the anatomical framework and physiological mechanisms regulating ANS function, thereby providing the backdrop for understanding autonomic pharmacodynamics and the development of specific autonomic drugs.

Anatomy of the Autonomic Nervous System: Overview

The ANS contains supraspinal, spinal, and peripheral (afferent and efferent) components, and is characterized by distinctive anatomical frameworks. The efferent components of both branches of the ANS use chemical neurotransmitters for conveying information between nerve cells (e.g., transmission from preganglionic neurons to postganglionic neurons at ganglionic sites) and from postganglionic neurons to target cells and tissues. Chemical neurotransmission involves the release of small amounts of neurotransmitter molecules from nerve terminals into the synaptic space, diffusion of transmitters across the synaptic space, neurotransmitter molecules binding to specialized receptors (specific receptors are discussed in a subsequent section) located primarily at postsynaptic sites on cells innervated by the ANS, and the subsequent activation or inhibition of intracellular secondary messenger mechanisms. The presence of adrenergic, cholinergic, and nonadrenergic–noncholinergic (NANC) neurons provides the essential substrates for generating and sustaining a diverse array of physiological functions. A fundamental tenet for understanding autonomic pharmacology begins with considering specific anatomical components of the sympathetic and parasympathetic nervous systems, which are described separately in this section.

Sympathetic Nervous System

Multiple sites in the central nervous system (CNS), including forebrain, brainstem, and spinal neural circuits, regulate the level of sympathetic nerve activity (Figure 6.2). The hypothalamus is a principal integration and control center of the ANS. Neurons within hypothalamic nuclei (e.g., the paraventricular nucleus) receive afferent signals from a variety of sources and, in turn, dispatch efferent signals to primary autonomic centers in the brainstem, (e.g., the rostral ventrolateral medulla (RVLM) and the midline medullary raphe nuclei). The axons of brainstem neurons within these centers (i.e., presympathetic neurons) project to sympathetic preganglionic neurons in the spinal cord (the axons of neurons in select hypothalamic nuclei also project to preganglionic neurons directly).

The cell bodies of sympathetic preganglionic neurons are located in the intermediolateral cell column of thoracic and cranial lumbar spinal segments (providing the derivation for the thoracolumbar terminology of the SNS). As mentioned, they are innervated by nerve endings of presympathetic neurons whose cell bodies are located in supraspinal nuclei. Sympathetic preganglionic nerve fibers exit the spinal cord in association with thoracic and lumbar spinal nerves and many sympathetic preganglionic neurons terminate and synapse in paravertebral ganglia located bilaterally on each side of the spinal column (Figures 6.1 and 6.5). Other sympathetic preganglionic neurons terminate and synapse in ganglia that are located closer to target organs, the prevertebral ganglia. Postganglionic sympathetic neurons arise from paravertebral and prevertebral ganglia and project to target organs and tissues (de Lahunta and Glass, 2009; Dyce et al., 2010; Evans and de Lahunta, 2013). The primary neurotransmitter released from preganglionic neurons at sympathetic ganglionic sites is acetylcholine (ACh), and at these sites ACh primarily binds to and activates nicotinic receptors located on the dendrites of postganglionic sympathetic neurons (Figure 6.5). Sympathetic ganglionic nicotinic receptors are classified as nicotinic neural (N_N) receptors. Nerve fibers that synthesize and release ACh are classified as cholinergic fibers; therefore sympathetic preganglionic nerve fibers are described as cholinergic. Norepinephrine (NE) (noradrenaline) is the predominant neurotransmitter released from postganglionic SNS neurons at target organ sites. At these sites NE primarily binds to and activates adrenergic receptors (designated as α and β receptors, as in Figure 6.5). Nerve fibers that synthesize and release NE are classified as adrenergic (or noradrenergic) fibers. Some postganglionic SNS neurons release dopamine (identified as dopaminergic neurons, as in Figure 6.5), which is the immediate metabolic precursor to NE and is an important neurotransmitter in the peripheral vasculature and

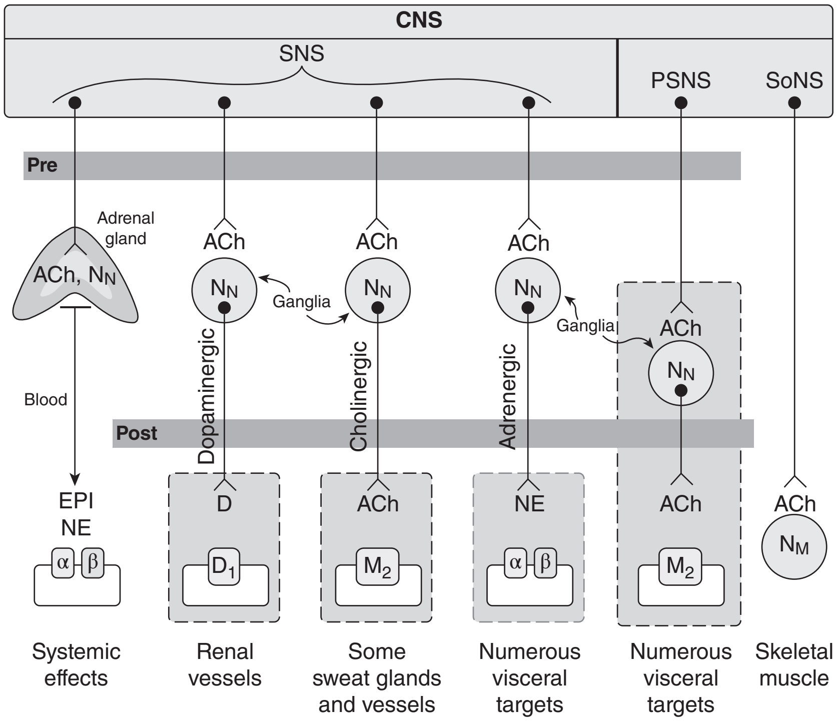

Figure 6.5 Schematic representation of the preganglionic and postganglionic anatomical relationships of nerves contained in the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS). The anatomical substrate of somatic motor nerves (SoNS) is also shown. Only the primary neurotransmitters are shown. ACh is the neurotransmitter released at sympathetic and parasympathetic ganglia and at most parasympathetic neuroeffector junctions. Norepinephrine (NE) is the primary neurotransmitter released at adrenergic sympathetic neuroeffector junctions (see text for exceptions.) "Visceral targets" refers to smooth muscle, cardiac muscle, and glands. Note that some sympathetic postganglionic fibers release acetylcholine or dopamine rather than NE. The adrenal medulla, a modified sympathetic ganglion, is innervated by sympathetic preganglionic fibers and releases epinephrine and NE into the blood. ACh, acetylcholine; D, dopamine; EPI, epinephrine; M, muscarinic receptors; N, nicotinic receptors; NE, norepinephrine.

the CNS (Figure 6.5). Dopamine binds to and activates dopaminergic receptors (designated as D_1, as in Figure 6.5). The chromaffin cells of the adrenal medulla are analogous to ganglionic neurons, are innervated by preganglionic SNS fibers and contain N_N receptors, and synthesize and release epinephrine (EPI) and NE (Figure 6.5). A subgroup of postganglionic SNS neurons release ACh as a neurotransmitter (such as the sympathetic innervation to sweat glands in humans and the sympathetic innervation of skeletal muscle arteries in some species), and these fibers are classified as postganglionic sympathetic–cholinergic fibers (Figure 6.5). The ACh released from postganglionic sympathetic–cholinergic fibers binds to and activates muscarinic receptors (designated as M_2 in Figure 6.5).

Along with NE, cotransmitter molecules or substances are released from many postganglionic SNS fibers at target tissues and organs (Gourine et al., 2009; Burnstock, 2013; Herring, 2015; Ralevic, 2015). ATP has been identified as a cotransmitter along with NE in sympathetic nerves. NE and ATP may be stored in the same vesicles and released together, or may be stored in separate vesicles and regulated as individual entities. The relative contributions of NE and ATP in cell-mediated physiological responses depends on the specific target tissue, species, and neural stimulation parameters. ATP mediates responses via activation of purinergic receptors that are identified as adenosine (P1) receptors and P2X and P2Y receptors. Neuropeptide Y (NPY) can also be released with NE from sympathetic nerves and is an agonist at neurokinin receptors (e.g., Y_1 and Y_2). The primary physiological role of this peptide is to modulate sympathetic neurotransmission at both pre- and postsynaptic sites. Cotransmitters have an important signaling role in the cardiovascular, urogenital, and respiratory systems.

Parasympathetic Nervous System

Like the SNS, multiple CNS sites are involved in regulating efferent parasympathetic nerve outflow, although the integrative central mechanisms are not as thoroughly defined. The hypothalamus is involved in parasympathetic integration and control, and hypothalamic nuclei communicate with brainstem autonomic centers (e.g., the pontine micturition center). The axons of brainstem neurons within these centers (i.e., preparasympathetic neurons) project to parasympathetic preganglionic neurons in the brainstem and spinal cord. The axons of neurons in hypothalamic nuclei may also project to preganglionic neurons directly.

The cell bodies of parasympathetic preganglionic neurons are located in brainstem nuclei (e.g., the parasympathetic nuclei of the oculomotor, facial, glossopharyngeal, and vagus nerves, as well as the nucleus ambiguus) (Figure 6.2), and in the intermediolateral cell column of the sacral spinal cord (Figure 6.1). As mentioned, they are innervated by nerve endings of preparasympathetic neurons whose cell bodies are found in supraspinal nuclei. Preganglionic axons exit the brainstem with their respective cranial nerves (preganglionic parasympathetic axons from the nucleus ambiguus join the vagus nerve) whereas they exit the sacral spinal cord in association with sacral spinal nerves. The specific placement of preganglionic PSNS cell bodies in the brainstem and sacral spinal cord provides the derivation for the craniosacral terminology of the PSNS. Most parasympathetic preganglionic neurons terminate and synapse in intramural ganglia (terminal ganglia) located within the innervated target organs. Other preganglionic parasympathetic fibers terminate and synapse in ganglia (e.g., ciliary, pterygopalatine, submandibular, otic, and pelvic ganglia) located outside the innervated target organs or on ganglion cells that are characterized by a rather diffuse distribution of cells located near target organs (de Lahunta and Glass, 2009; Dyce et al., 2010; Evans and de Lahunta, 2013). Like the SNS, the primary neurotransmitter released from preganglionic neurons at parasympathetic ganglionic sites is ACh, and at these sites ACh primarily binds to and activates nicotinic receptors located on postganglionic parasympathetic neurons (Figure 6.5). Parasympathetic ganglionic nicotinic receptors are classified as N_N receptors. Postganglioinic parasympathetic neurons arise from parasympathetic ganglia and project to target cells, tissues, and organs. ACh is also the primary neurotransmitter released from postganglionic PSNS neurons at target organ sites, and at these sites ACh primarily binds to and activates muscarinic receptors (designated as M_2 receptors, as in Figure 6.5). As stated previously, nerve fibers that synthesize and release ACh are classified as cholinergic fibers, therefore both preganglionic and postganglionic parasympathetic neurons are cholinergic.

It is common that cotransmitters are released, along with ACh, from many postganglionic PSNS fibers at target tissues and organs (Gourine et al., 2009; Burnstock, 2013; Herring, 2015; Ralevic, 2015). Parasympathetic nerves innervating the urinary bladder release ATP as a cotransmitter and there is evidence that parasympathetic nerves supplying the salivary glands utilize vasoactive intestinal polypeptide as a cotransmitter. Similar to sympathetic nerves, ACh and specific cotransmitters may be stored in the same vesicles and released together, or may be stored in separate vesicles and regulated as individual entities. The relative contributions of ACh, ATP, vasoactive intestinal polypeptide, and other putative neurotransmitters and cotransmitters in cell-mediated physiological responses depends on the specific target tissue, species, and neural stimulation parameters.

Nonadrenergic–Noncholinergic Neurons

Some nerves that project to and innervate effector tissues and organs, generally at anatomical sites that are innervated by autonomic nerves, do not exhibit the histochemical characteristics of cholinergic or adrenergic fibers. These neurons are classified as nonadrenergic–noncholinergic (NANC) fibers. Many of the same molecules/substances identified as cotransmitters in ANS nerves also play a role as neurotransmitters released from NANC nerve endings (e.g., ATP, NPY) (Gourine et al., 2009; Burnstock, 2013; Herring, 2015; Ralevic, 2015). Substantial evidence points to an important role of NANC transmission in the physiological regulation of the GI tract (the enteric nervous system is prominently innervated by cholinergic, adrenergic, and NANC nerve fibers), genitourinary tract, and select blood vessels. For example, nitric oxide (NO), synthesized and released from NANC nerves and endothelial cells, is an important contributor to penile erection (Burnett, 2006; Lasker et al., 2013). NO is produced in NANC neurons by neural NO synthase (nNOS) and in endothelial cells by endothelial NO synthase (eNOS). NO is considered a primary vasoactive neurotransmitter and chemical mediator, and after binding to an intracellular receptor results in the conversion of GTP to cGMP. ACh released from cholinergic nerves can bind to muscarinic receptors on endothelial cells and initiate the formation of NO. NANC neurons may also contain extensive afferent components, which are thought to contribute to the local, reflex regulation of sensory inputs.

Enteric Nervous System

Regulation of the complex physiological processes associated with gastrointestinal physiology, including secretions, motility, and nutrient absorption, are mediated to a large extent by the enteric nervous system, an intrinsic nervous system located in the wall of the gastrointestinal system. The enteric nervous system contains both motor and sensory components and is characterized by the presence of a complex intrinsic neural network that includes the myenteric plexus and the submucosal plexus. Afferent information from nerve endings in the gut wall and mucosa convey chemical, mechanical, and stretch sensory information to the intrinsic neural networks. The enteric neural networks are innervated by preganglionic PSNS nerve fibers and postganglionic SNS nerve fibers. The parasympathetic and sympathetic innervation to the enteric nervous system appears to influence the intrinsic function of this nervous system in a modulatory manner. Sensory fibers in the enteric nervous system also transmit information to sympathetic ganglia and can modulate postganglionic sympathetic nerve outflow (Evans and de Lahunta, 2013). The enteric nervous system is extensively innervated by NANC nerve fibers.

Integrated Steps in SNS and PSNS Neurotransmission

The physiology of ANS function and regulation involves numerous integrated steps, including: regulation of supraspinal and spinal autonomic neural circuits; changing the level of efferent activity in sympathetic and parasympathetic nerves that innervate peripheral organs and tissues; neurotransmitter and neuromodulator synthesis, release, and degradation; chemical transmission from nerve cell to nerve cell (e.g., ganglionic regulation); chemical transmission from nerve cells to target organ sites; receptor-mediated effects; and regulation of activity in afferent nerves. Each of these processes and functions can be manipulated by pharmacological agents, providing multiple sites for drug-mediated changes in ANS regulation and function.

Peripheral Nerve Activity and Transmission of Action Potentials

Central neural circuits are critically involved in generating the basal activity in peripheral sympathetic and parasympathetic nerves, and in altering the level of activity in these nerves in response to internal and external stimuli. The arrival of nerve impulses at axonal terminals initiates a series of physiological processes that culminate with the release of neurotransmitters and cotransmitters into the synaptic cleft. An in-depth discussion regarding fundamental physiological processes involved in the conduction of action potentials along nerve axons, including resting membrane potential, changes in membrane ionic conductances, membrane depolarization, action potential propagation, and membrane repolarization, is beyond the scope of this introductory chapter. Generally, axonal conduction is not markedly influenced by pharmacological interventions, although there are several key exceptions. For example, voltage-sensitive membrane sodium channels are blocked and axonal conduction is inhibited by local anesthetics such as lidocaine, and by tetrodotoxin (puffer fish toxin) and saxitoxin (shellfish toxin).

Neurotransmitter Synthesis, Storage, Release, and Inactivation

Cholinergic Neurons

Cholinergic nerve terminals contain several types of vesicles, including small membrane-bound vesicles that play a key role in ACh storage (Figure 6.6), and larger vesicles that contain biological substances (e.g., ATP) that are cotransmitters released simultaneously along with ACh from cholinergic nerves (Table 6.2).

The synthesis of vesicles is typically completed in neuronal cell bodies and vesicles are relocated to nerve terminals by axonal transport mechanisms. Vesicles contain numerous proteins involved in transport and trafficking processes and an extensive amount of research has been focused on understanding these cellular processes. One important functional group of proteins are termed vesicle-associated membrane proteins (VAMPs), which play a critical role in aligning ACh-containing vesicles with functional release sites on the inner neuronal cell membrane, and in initiating neurotransmitter release. Release sites on the presynaptic plasma membrane contain synaptosomal nerve-associated proteins (SNAPs), which interact with vesicle-associated membrane proteins to facilitate vesicle–plasma membrane fusion and subsequently neurotransmitter release (Figure 6.6). Botulinum toxin interferes with ACh release from cholinergic nerve terminals.

The enzyme choline acetyltransferase (ChAT) synthesizes ACh from acetyl-CoA and choline in the cytoplasm of cholinergic nerves (Figure 6.6). Neurons in the PSNS are characterized by high rates of ACh release, which is mediated by a highly efficient and rapid process of ACh synthesis. Choline is transported across the neuronal membrane from the extracellular space via a sodium-dependent membrane choline transporter (CHT; Figure 6.6), whereas acetyl-CoA is synthesized in mitochondria located in the nerve endings. Choline transport into the cell can be limited by blocking the choline transporter using a group of research drugs called hemicholiniums. Following synthesis in the cytoplasm, ACh is transported into vesicles by a vesicle-associated transporter, which can be antagonized by the research drug vesamicol. Acetylcholine storage in vesicles is characterized by the packaging of "quanta" of ACh molecules (normally 1,000 to 50,000 molecules in each vesicle).

Release of ACh neurotransmitter from vesicles in cholinergic nerves is dependent on the arrival of action potentials at neuronal membrane terminals which activate voltage-gated calcium channels allowing for inward movement of Ca^{2+} into the nerve terminal (Figure 6.6). Calcium initiates processes that are essential for the vesicle membrane fusion with the neuronal membrane, leading to the exocytotic discharge of neurotransmitter into the synaptic cleft. Following release of ACh from cholinergic nerve terminals, ACh molecules may bind to and activate nicotinic (primarily ganglionic sites in the ANS) and muscarinic (primarily cells and tissues at target sites innervated by postganglionic nerves) receptors. Atropine and related alkaloids are muscarinic receptor antagonists whereas chlorisondamine blocks N_N receptors.

Nicotinic and muscarinic receptors are also present at presynaptic sites. Following release of ACh from cholinergic nerve terminals, this neurotransmitter can

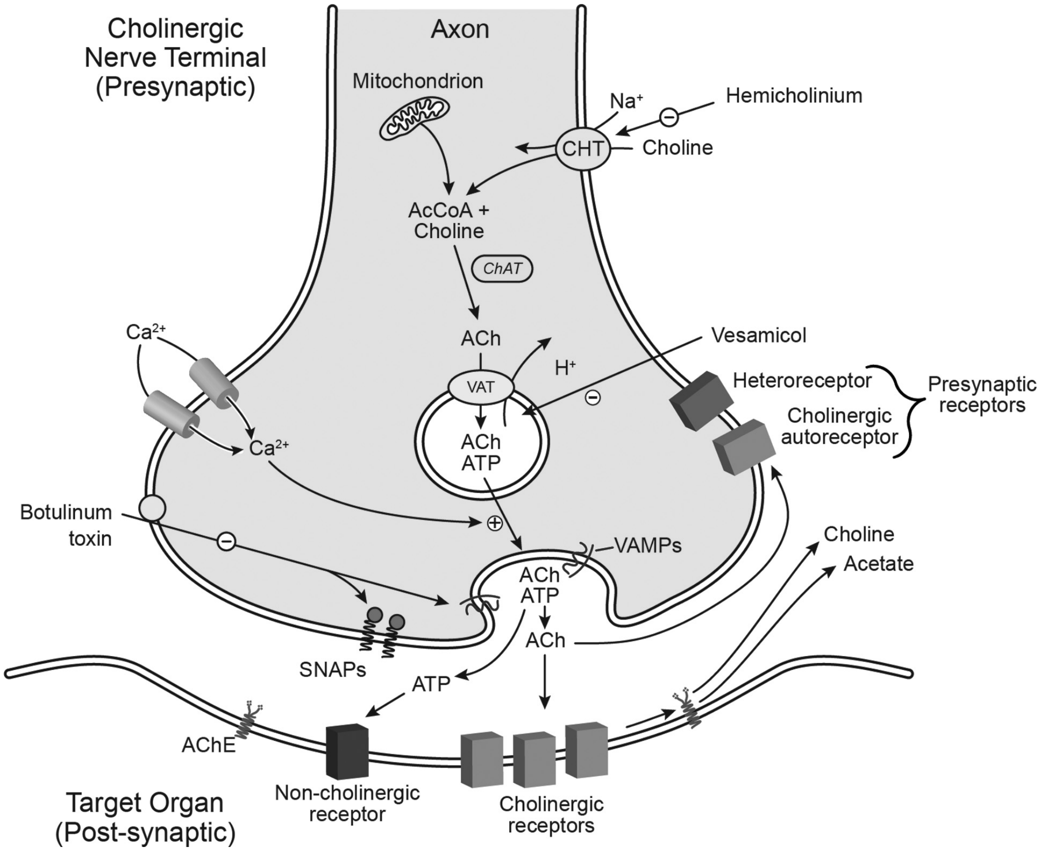

Figure 6.6 Schematic diagram depicting physiological processes at the site of a postganglionic parasympathetic nervous system neuron innervating a target tissue. Processes are described in the text and include: neurotransmitter synthesis, storage, and release; chemical transmission from nerve cells to target organ cells and tissues; neurotransmitter activation of presynaptic receptors; and termination of neurotransmitter action.

bind to and activate presynaptic cholinergic autoreceptors, primarily nicotinic receptors on preganglionic neurons and muscarinic receptors on postganglionic neurons, which in turn attenuate the release of ACh. This physiological process provides a pathway via which the release of ACh from a specific cholinergic neuron is regulated at a local level, an autoregulatory process. Cholinergic receptors also function as heteroreceptors, that is ACh released from cholinergic nerve terminals can bind to nicotinic or muscarinic receptors located on the presynaptic terminals of adjacent neurons, such as sympathetic nerve terminals, and inhibit the release of neurotransmitters from these neurons, such as NE from postganglionic sympathetic neurons.

As stated previously, cotransmitter molecules or biological substances are released along with ACh at target sites innervated by postganglionic parasympathetic neurons. Physiological functions of PSNS cotransmitters include modulating the presynaptic release of ACh, producing synergistic actions with ACh, and activating different postjunctional cells. In general, PSNS cotransmission at synaptic sites provides increased pharmacological and physiological finesse in the regulation of target tissue responses.

The binding of ACh to cholinergic receptors is transient and ACh located in the synaptic space is inactivated by acetylcholinesterase (AChE) (Figure 6.6), an enzyme that rapidly hydrolyzes ACh into choline and acetate. AChE is located in close proximity to the synaptic cleft at most cholinergic synapses, including postganglionic parasympathetic neuroeffector sites, and is present in autonomic ganglia, cholinergic nerves, and at neuromuscular junctions. The distribution of ACh released from cholinergic neurons is localized because AChE is efficient at terminating the action of ACh at synaptic sites. The amount of ACh available at synaptic sites, and subsequently its functional activity, can be enhanced by drugs and chemicals that block AChE (e.g., acetylcholinesterase agents). AChE is found in other body tissues, including red blood cells.

Adrenergic Neurons

Many animal species synthesize and secrete three endogenous catecholamines: NE, EPI, and dopamine. Biochemical steps in the synthesis of catecholamines are shown in Figure 6.7. Catecholamine synthesis is initiated by the conversion of phenylalanine to tyrosine by the enzyme phenylalanine hydroxylase. The enzyme tyrosine

Table 6.2 Autonomic nervous system receptor types, typical anatomical locations, and their primary mechanisms of action

Receptor name	Typical locations/tissue	G-Protein	Cellular responses
Cholinoceptors			
Muscarinic M_1	CNS; enteric nervous system; glands; sympathetic postganglionic neurons	$G_{q/11}$	Activation of PLC, IP_3 and DAG, ↑ intracellular calcium
Muscarinic M_2	CNS; heart; smooth muscle; select presynaptic sites	$G_{i/o}$	Activation of K^+ channels, adenylyl cyclase inhibition, and ↓ cAMP
Muscarinic M_3	CNS; heart; glands; smooth muscle	$G_{q/11}$	Activation of PLC, IP_3 and DAG
Muscarinic M_4	Prominently expressed in the CNS; vagal nerve terminal	$G_{i/o}$	Activation of K^+ channels, adenylyl cyclase inhibition, and ↓ cAMP
Muscarinic M_5	CNS; vascular endothelium	$G_{q/11}$	Activation of PLC, IP_3 and DAG
Nicotinic N_N	SNS and PNS autonomic ganglia; postganglionic neurons; presynaptic nerve terminals		Depolarization of postganglionic SNS and PNS neurons, ↑ ionic permeability (Na^+, K^+)
Nicotinic N_M	Somatic nervous system; neuromuscular junction		Depolarization and skeletal muscle contraction, ↑ cation permeability
Adrenoceptors			
α_{1A}	Vascular smooth muscle; urogenital smooth muscle; reproductive organs; CNS	G_q	Activation of PLA_2, PLC, IP_3 and DAG, ↑ intracellular calcium
α_{1B}	Vascular smooth muscle; spleen; liver; CNS	G_q	Activation of PLA_2, PLC, IP_3 and DAG, ↑ intracellular calcium
α_{1D}	Platelets; CNS	G_q	Activation of PLA_2, PLC, IP_3 and DAG, ↑ intracellular calcium
α_{2A}	Presynaptic adrenergic nerve terminals; CNS; brainstem and spinal cord sites; postganglionic SNS neurons; autonomic ganglia; platelets	G_i/G_o	Inhibition of adenylyl cyclase, ↓ cAMP
α_{2B}	Vascular smooth muscle; CNS; liver; kidney	G_i/G_o	Inhibition of adenylyl cyclase, ↓ cAMP
α_{2C}	CNS	G_i/G_o	Inhibition of adenylyl cyclase, ↓ cAMP
β_1	Heart; kidney; juxtaglomerular cells; CNS; presynaptic sites on adrenergic and cholinergic nerve terminals	G_s	Stimulation of adenylyl cyclase, ↑ cAMP
β_2	Smooth muscle (bronchial, vascular, bladder); heart; liver; skeletal muscle	G_s	Stimulation of adenylyl cyclase, ↑ cAMP
β_3	Adipose tissue	G_s	Stimulation of adenylyl cyclase, ↑ cAMP
Dopamine receptors			
D_1 (DA_1), D_5	CNS; kidney; vascular smooth muscle	G_s	Stimulation of adenylyl cyclase, ↑ cAMP
D_2 (DA_2), D_3, D_4 D_5	CNS; smooth muscle; presynaptic nerve terminals	G_i/G_o	↓ Intracellular cAMP; ↑ K^+ currents

CNS, central nervous system; SNS, sympathetic nervous system; PNS, parasympathetic nervous system; PLC, phospholipase C; PLA, phospholipase A; IP_3, inositol-1, 4, 5-triphosphate; DAG, diacylglycerol; cAMP, cyclic AMP.

hydroxylase converts tyrosine to dihydroxyphenylalanine (dopa), and this enzymatic step is considered the rate-limiting step in catecholamine synthesis. Dopa is decarboxylated by the enzyme L–aromatic amino acid decarboxylase (dopa decarboxylase) to form dihydroxyphenylethylamine (dopamine), which is transported into and stored in granules. In some neurons and neuronal systems, including select peripheral postganglionic sympathetic neurons and neurons in several CNS sites (e.g., mammalian extrapyramidal system), catecholamine synthesis terminates with dopamine. These neurons release dopamine as the primary neurotransmitter and are identified as dopaminergic neurons. Dopamine–β-hydroxylase mediates the conversion of dopamine to NE, and this conversion is the terminal enzymatic step in catecholamine synthesis in postganglionic SNS neurons and in some CNS neurons. The chromaffin cells of the adrenal medulla synthesize and release EPI and NE. In some adrenal medullary cells the terminal step in the biosynthesis of catecholamines involves conversion of dopamine to NE, whereas in other cells NE is released from the granules and is converted to EPI within the cytoplasm by phenylethanolamine *N*-methyltransferase, and subsequently re-enters the chromaffin granules prior to release from the adrenal medulla. EPI (~80%; NE, ~20%) is the primary catecholamine released from the adrenal medulla. The enzymatic conversion of NE to EPI also occurs in select CNS sites. The level of catecholamines contained in the cytoplasm is closely regulated by two physiological

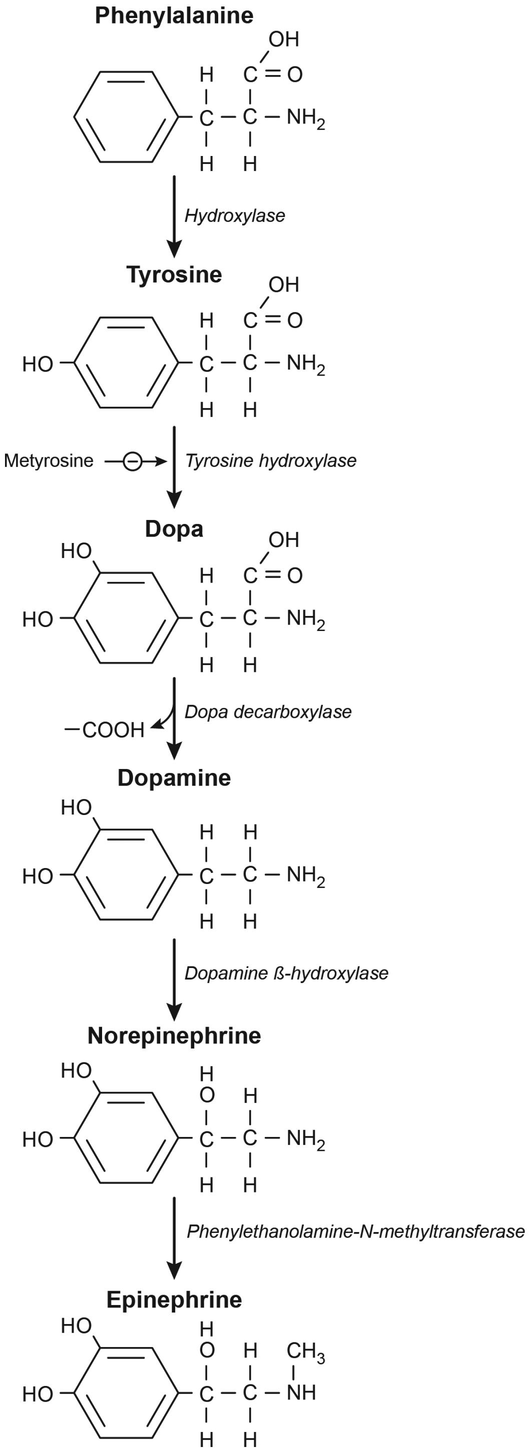

Figure 6.7 Biosynthesis of catecholamines. The rate-limiting step is the conversion of tyrosine to dopa by the enzyme tyrosine hydroxylase. Generally, postganglionic sympathetic nervous system neurons do not contain the enzyme, phenylethanolamine-*N*-methyltransferase (PMNT), therefore, the enzymatic conversion of dopamine to norepinephrine is the final step in catecholamine biosynthesis in these neurons. The enzymatic conversion of norepinephrine to epinephrine by PMNT occurs primarily in the adrenal medulla and in specific central nervous system sites.

processes: inactivation by the neuronal mitochondrial enzyme monoamine oxidase (MAO); and transportation of cytoplasmic NE into granules by the vesicular monoamine transporter. NE, EPI, dopamine, and serotonin are all transported by monoamine transporters and reserpine inhibits this process and leads to depletion of the amount of stored neurotransmitter.

The exocytic process of releasing neurotransmitters and cotransmitters from granules contained in adrenergic nerve endings is initiated by arrival of an action potential at the nerve terminal (Figure 6.8), is calcium dependent, and is similar in many aspects to the release of ACh from cholinergic nerves. Although NE is the primary neurotransmitter released from postganglionic sympathetic nerves, there are several cotransmitters that can be simultaneously released, including ATP, NPY, and other peptide molecules. These additional neurotransmitters or neuromodulators may be costored with NE or may be stored in separate vesicles. The functions of cotransmitters in synaptic neurotransmission are varied and multifactorial and may include direct postsynaptic receptor effects, modulation of receptor effects of other adrenergic neurotransmitters or cotransmitters, and alteration of the presynaptic release of neurotransmitters or neuromodulators.

Following release from postganglionic sympathetic nerve terminals, NE molecules can bind to and activate adrenergic receptors located on postsynaptic cells and tissues, as well as activate presynaptic adrenergic receptors located on the postganglionic nerve terminal from which the catecholamine was released (Figure 6.8). α_2-adrenergic receptors (with a specific emphasis on the α_{2A}-adrenergic receptor subtype) are the primary presynaptic receptors that, when activated, mediate an autoregulatory response to inhibit transmitter release at selected postganglionic SNS nerve terminals. The release of NE from postganglionic SNS neurons can also be reduced by several of the cotransmitters that are released simultaneously with NE. Conversely, the release of NE from postganglionic SNS neurons can be augmented by activation of presynaptic β_{2}-adrenergic receptors. Adrenergic receptors located on the presynaptic terminals of adjacent neurons, such as nearby parasympathetic nerve terminals, can function as heteroreceptors. For example, NE released from postganglionic sympathetic nerve terminals can bind to adrenergic receptors located on parasympathetic nerve terminals and inhibit ACh release. Similarly, the activation of heteroreceptors located on sympathetic nerve terminals, such as the binding of ACh to muscarinic receptors expressed on postganglionic SNS neurons, can inhibit the release of catecholamines from sympathetic nerve terminals.

As a physiological strategy to reduce overstimulation of adrenergic receptors secondary to neuronal release

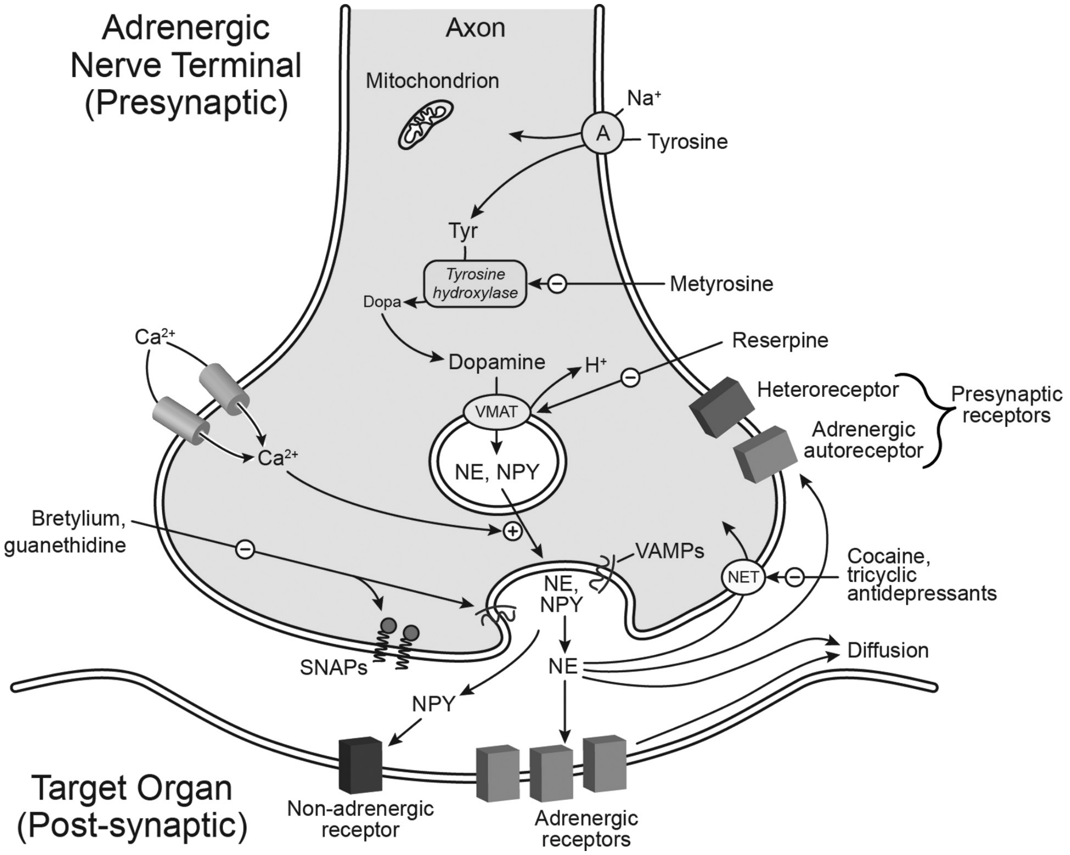

Figure 6.8 Schematic diagram depicting physiological processes at the site of a postganglionic sympathetic nervous system neuron innervating a target tissue. Processes are described in the text and include: neurotransmitter synthesis, storage, and release; chemical transmission from nerve cells to target organ cells and tissues; neuronal reuptake of neurotransmitter; neurotransmitter activation of presynaptic receptors; and termination of neurotransmitter action.

of NE, the amount of NE contained in the synapse is tightly regulated. Elimination of synaptic NE and/or termination of NE synaptic activity occurs via three physiological processes. First, NE can be metabolized by the enzyme catechol-*O*-methyltransferase (COMT) that is located at postsynaptic cell membrane-associated neuroeffector sites. Second, NE (as well as other similar molecules) can be transported back into postganglionic sympathetic nerve terminals (or nearby glial cells) by an active norepinephrine transporter (NET, also termed uptake 1 or reuptake 1) reuptake system that is present in axonal membranes (Figure 6.8). In a similar process, synaptic dopamine can be taken back into dopaminergic cells by a membrane dopamine transporter. NE that has been transported back into the neuron may be recycled and placed in granules or undergo metabolic breakdown via mitochondrial MAO. Inhibition of NET, mediated by cocaine and tricyclic antidepressant drugs, results in an increase in the level of NE in the synaptic cleft and enhanced receptor-mediated effects. Third, NE can diffuse away from the synaptic cleft and be metabolized in the plasma or liver, or excreted in the urine. Hepatic COMT is critically involved in the metabolism of endogenous circulating and administered catecholamines. It is generally considered that the reuptake of synaptic NE into adrenergic nerve terminals and the diffusion of NE from synaptic areas and subsequent enzymatic degradation are the primary processes involved in terminating NE activity. Metabolism of endogenous catecholamines by MAO and COMT involves numerous pathways (Figure 6.9).

Autonomic Receptors

Multiple experimental interventions (including structure–activity analyses, binding of isotope-labeled ligands, pharmacokinetic and pharmacodynamic analyses of specific autonomic agonists and antagonists, and molecular biological and proteomic analyses) have been used to identify and characterize receptors involved in ANS regulation. These include nicotinic and muscarinic cholinergic receptors, as well as alpha, beta, and dopaminergic adrenergic receptors (Wilson-Pauwels et al., 1997; Yamada and Ito, 2001; Abrams and Andersson, 2007; Yoshimura et al., 2008; Andersson,

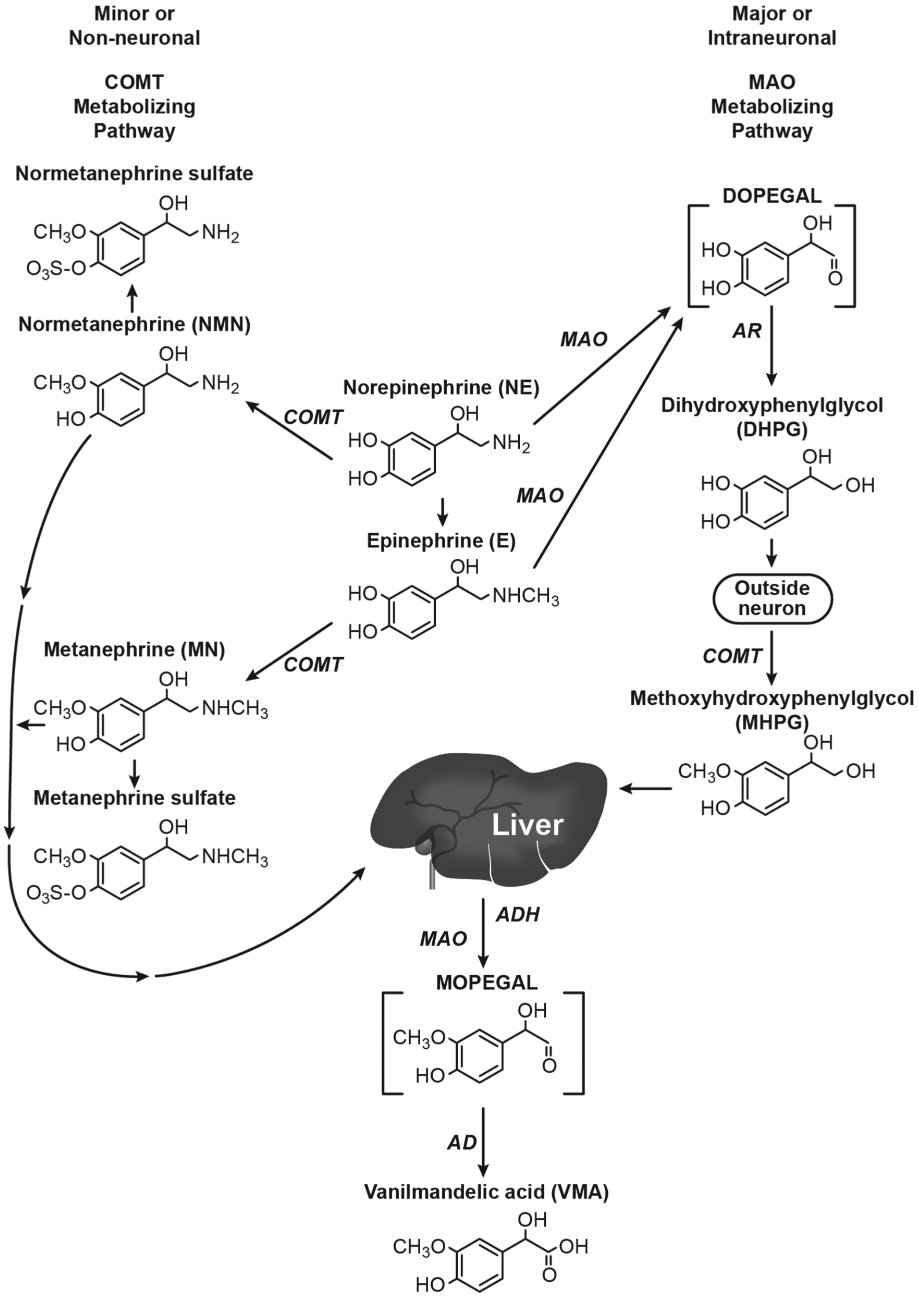

Figure 6.9 Flow diagram illustrating the metabolism of norepinephrine and epinephrine by catechol-*O*-methyltransferase (COMT) and monoamine oxidase (MAO). Source: Adapted from Oeltmann 2004. Reproduced with permission of Elsevier.

2011; Cazzola et al., 2012; Lei, 2014; Herring, 2015). Specific receptor information is summarized in Table 6.2 (receptors/sites/G proteins/secondary messengers) and described in subsequent sections.

Cholinergic Receptors

As stated previously, ACh is the primary agonist at two types of cholinergic receptors (the term cholinoceptor is often used interchangeably): nicotinic and muscarinic. These receptors were named according to the alkaloids (nicotine and muscarine) that were identified to be agonists at the respective receptors.

Nicotinic Receptors: Anatomical Location, Receptor Subtypes, and Signal Transduction

Peripherally located autonomic N_N receptors are present on postganglionic neurons in autonomic ganglia (intramural, prevertebral, and paravertebral) and mediate neurotransmission from preganglionic to postganglionic neurons in both arms of the ANS. N_N receptors are also present on adrenal medullary chromaffin cells and mediate neurotransmission from preganglionic SNS neurons to adrenal medullary chromaffin cells. Nicotinic receptors are widely distributed in the CNS and are present in nonneuronal tissues. Nicotinic muscle (N_M) receptors are critically involved in mediating signal transmission at the neuromuscular junction and are an essential component of the somatic nervous system (SoNS) (Figure 6.5).

Nicotinic receptors are ligand-gated ion channels and contain five homologous subunits organized around a central pore (Stokes et al., 2015). Activation of these receptors initiates a rapid increase in cellular permeability to selective cations (Na^+ and Ca^{2+}); cell membrane depolarization; and excitation of postganglionic ANS neurons, adrenal medullary chromaffin cells, or skeletal muscle fibers (Stokes et al., 2015).

As previously discussed, in addition to their anatomical location at postsynaptic sites on effector cells, nicotinic receptors are located at presynaptic sites. Activation of these receptors influences the release of neurotransmitters from neurons at peripheral sites and in the CNS.

Muscarinic Receptors: Anatomical Location, Receptor Subtypes, and Signal Transduction

Muscarinic receptors are located predominately at postsynaptic target sites innervated by postganglionic parasympathetic nerves such as the heart, glands, urinary bladder, and gastrointestinal tract, thereby establishing a pivotal role for these receptors in the functionality of the PSNS. Five subtypes of muscarinic receptors have been identified, each associated with a different gene, and many of the physiological functions associated with PSNS activation are mediated by muscarinic$_2$ (M_2) and muscarinic$_4$ (M_4) receptors. Muscarinic receptor subtypes are located in distinct peripheral anatomic locations (Table 6.2) and demonstrate differential specificities to various agonists and antagonists.

Muscarinic receptors are G protein-coupled receptors (GPCRs), and activation of these receptors may elicit an excitatory or inhibitory response (Calebiro et al., 2010; Jalink and Moolenaar, 2010; Ambrosio et al., 2011; Vischer et al., 2011; Latek et al., 2012; Duc et al., 2015). A fundamental mechanism mediating the capability of the PSNS to produce an assortment of physiological response profiles arises from the fact that specific muscarinic receptors couple primarily to specific G proteins. Muscarinic receptor subtypes M_1, M_3, and M_5 couple through $G_{q/11}$, whereas M_2 and M_4 receptors couple to G_i and $G_{0.}$ Specificity in the intracellular response profiles following activation of specific muscarinic receptors are the result of G protein-mediated effects on the generation of second messengers and on the activity of ion channels (Table 6.2).

As described previously, and in addition to their anatomical location at postsynaptic sites on effector cells innervated by postganglionic PSNS neurons, muscarinic receptors are located at presynaptic and perisynaptic sites and activation of these receptors influences the release of neurotransmitters from neurons at peripheral sites and in the CNS.

Adrenergic Receptors

The endogenous catecholamines NE and EPI are agonists at α- and β-adrenergic receptors (also termed adrenoceptors), whereas dopamine, the metabolic precursor of NE, is a primary agonist at dopaminergic receptors.

Adrenergic Receptors: Anatomical Location, Receptor Subtypes, and Signal Transduction

The concept of distinct adrenergic receptors (α and β) as determined by their relative responsiveness to specific receptor agonists was first proposed in a classic paper authored by Ahlquist (1948). There are two types of α-adrenergic receptors; α_1 and α_2. Each type contains specific receptor subtypes, designated as α_{1A}, α_{1B}, and α_{1D}; and α_{2A}, α_{2B}, and α_{2C}. There are three primary types of β-adrenergic receptors, β_1, $\beta_{2,}$ and $\beta_{3,}$ and two primary types of dopaminergic receptors, dopamine$_1$, D_1, and dopamine$_2$, D_2.

α- and β-adrenergic receptors are expressed predominately at target sites innervated by postganglionic sympathetic nerves, and their placement is characterized by a substantial degree of anatomical specificity (adrenergic types and designated tissue locations are summarized in Table 6.2). β-adrenergic receptors are expressed in the heart (primarily β_1), urinary bladder (primarily β_2 and β_3), liver (primarily β_2), kidney (primarily β_1); and in bronchial (primarily β_2), uterine (primarily β_2 and β_3), and vascular (primarily β_2) smooth muscle. It is often considered that vascular smooth muscle β_2-adrenergic receptors are not innervated by postganglionic sympathetic nerve fibers and circulating catecholamines released from the adrenal medulla are the primary endogenous agonists for these receptors. β-adrenergic receptors regulate many physiological functions including: heart rate and cardiac contractility; renin release; smooth muscle relaxation; and numerous metabolic events in adipose, skeletal muscle, and hepatic cells (see Table 6.1). α_1-adrenergic receptors are expressed in numerous tissues and organs including vascular smooth muscle, radial muscle of the iris, and smooth muscle in the genitourinary system. It is generally considered that α_1-adrenergic receptors are in close proximity to postganglionic sympathetic nerve endings, and NE released from these neurons is a primary endogenous agonist for these receptors. α_2-adrenergic receptors are expressed in a variety of cells and tissues including: vascular smooth muscle; thromobocytes; endothelial cells that synthesize and release nitric oxide; CNS sites; and on the terminals of postganglionic sympathetic nerve fibers.

Adrenergic receptors (α and β) are G protein-coupled receptors that link to G proteins (Calebiro et al., 2010; Jalink and Moolenaar, 2010; Ambrosio et al., 2011; Vischer et al., 2011; Latek et al., 2012; Duc et al., 2015).

A fundamental mechanism underpinning the capability of the ANS to produce various types of physiological response profiles arises from the fact that specific adrenergic receptors couple primarily to specific G proteins. For example, α_1 receptors couple to G_q, α_2 receptors couple to G_i, and β-adrenergic receptors couple to G_s. Specificity in the intracellular response profiles following activation of specific adrenergic receptors are the result of G protein-mediated effects on the generation of second messengers and on the activity of ion channels (Table 6.2).

As stated above, α_2-adrenergic receptors are expressed on the terminals of sympathetic nerve fibers, and NE released from postganglionic sympathetic neurons may interact with α_2-adrenergic receptors located at presynaptic sites to reduce NE release. The α_{2A}-adrenergic receptor subtype is considered to play an important role in this autoregulatory function. α-adrenergic receptors, especially α_2 receptors, can also be classified as heteroreceptors. For example, NE released from postganglionic sympathetic nerve fibers can modulate ACh release by activating α_2-adrenergic receptors that are expressed on parasympathetic neurons.

Dopamine mediates numerous physiological effects in the CNS and at peripheral targets, including the vasculature in many visceral organs. The D_1 receptor is generally associated with stimulation of adenylyl cyclase and increased cAMP, which may mediate the vasodilatory effects of dopamine in the renal and splanchnic circulations. On the other hand, activation of D_2 receptors has been shown to inhibit adenylyl cyclase activity, open potassium channels, and decrease calcium influx. D_2 receptors are expressed on the terminals of postganglionic sympathetic nerve fibers, and activation of these presynaptic dopaminergic receptors can produce the inhibition of NE from these nerve terminals.

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7

Adrenergic Receptor Agonists and Antagonists

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Classification of Adrenergic Agonists

The sympathetic nervous system (SNS) influences the regulation of most organ systems and plays a critical role in regulating physiological homeostasis under basal conditions and in response to acute and sustained stressors. Changes in physiological function are initiated and sustained by altering the level of efferent sympathetic nerve outflow, which in turn affects the release of norepinephrine (NE) from postganglionic nerve terminals. Sympathetic nerve activity mediates NE release from nerve terminals, and the released NE binds to and activates adrenergic receptors located on postsynaptic effector tissues and at presynaptic sites. In a similar fashion, central activation of sympathetic nerve activity directed to the adrenal medulla initiates the release of epinephrine (EPI) and some NE from this gland, which circulates in the blood and binds to and activates adrenergic receptors in target tissues.

Drugs that mimic the pharmacological and physiological actions of the endogenous catecholamines are classified as sympathomimetic drugs, that is their effects are mediated by activation of adrenergic receptors located on effector cells and tissues. One framework for classifying the functionality of adrenergic receptor agonists is centered on their general pharmacological mechanism of action (Figure 7.1). Three classifications are often considered. The first group, which includes the endogenous catecholamines (e.g., NE and EPI) and many sympathomimetic drugs (e.g., phenylephrine, dobutamine), are classified as direct agonists. These neurotransmitters and drugs bind directly to and activate adrenergic receptors (Figure 7.1). Many adrenergic agonists used in clinical medicine are direct adrenergic receptor agonists. A second group is classified as indirect-acting agonists because they mediate physiological responses via a pharmacological mechanism of action that involves increasing the synaptic levels of endogenous catecholamines, thereby enhancing the availability of endogenous catecholamines to bind to adrenergic receptors. This effect can be achieved by three different mechanisms of action: (i) reducing the metabolic breakdown of catecholamines by pharmacologically blocking or antagonizing endogenous enzymes involved in the metabolism of NE and EPI (e.g., monoamine oxidase inhibitors); (ii) inhibiting the physiological processes involved in the reuptake of NE from the synaptic space into postganglionic sympathetic nerve terminals (e.g., cocaine and tricyclic antidepressants); and (iii) enhancing the release of catecholamines from postganglionic sympathetic nerve terminals (e.g., tyramine). Some drugs (e.g., ephedrine) demonstrate the capability to directly activate adrenergic receptors as well as augment the release of NE from adrenergic nerve terminals, and these drugs are classified as mixed-acting adrenergic agonists (Figure 7.1). In a conceptual context, the physiological responses produced by direct, indirect, and mixed-acting agonists are similar to responses produced by activating central sympathetic neural circuits, increasing the level of efferent sympathetic nerve outflow, and inducing the release of NE from postganglionic sympathetic nerves.

Sympathomimetic Amines and Structure–Activity Relationships

The capacity of sympathomimetic drugs to produce physiological responses consistent with functional patterns produced by SNS activation depends on the similarity of the chemical structure of sympathomimetic drugs to that of NE and EPI. The parent compound of sympathomimetic amines is β-phenylethylamine, which consists of a benzene ring and an ethylamine side chain. Chemical substitutions can be made on the aromatic ring, on the α and β carbons atoms, and on the terminal amino group to produce compounds with sympathomimetic activity.

The endogenous adrenergic receptor agonists EPI, NE, and dopamine, as well as the synthetic sympathomimetic isoproterenol, contain a hydroxyl group on the 3 and 4 positions of the benzene ring. The 3,4-dihydroxybenzene structure is also known as catechol; therefore sympathomimetic amines that contain this nucleus are known as

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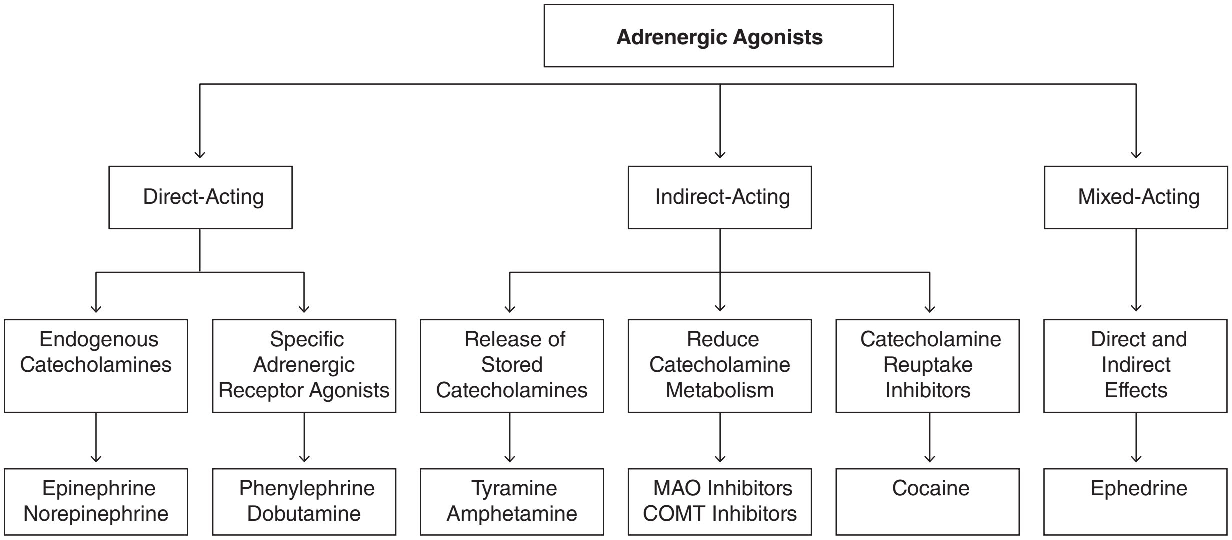

Adrenergic Agonists
Direct-Acting
Indirect-Acting
Mixed-Acting
Endogenous Catecholamines
Specific Adrenergic Receptor Agonists
Release of Stored Catecholamines
Reduce Catecholamine Metabolism
Catecholamine Reuptake Inhibitors
Direct and Indirect Effects
Epinephrine Norepinephrine
Phenylephrine Dobutamine
Tyramine Amphetamine
MAO Inhibitors COMT Inhibitors
Cocaine
Ephedrine

Figure 7.1 Framework for classifying the functionality of catecholamines and sympathomimetics based on pharmacological mode of action, that is direct agonists, indirect agonists, and mixed agonists. COMT, catechol-*O*-methyltransferase; MAO, monoamine oxidase.

catecholamines. For the most part, the catechol nucleus is required for maximum α- and β-adrenergic receptor potencies, and modifications in phenylethylamine mediate changes in the affinity of drugs for specific adrenergic receptors, and affect the intrinsic ability to activate these receptors. Removal of one or both hydroxyl groups from the aromatic ring reduces β-adrenergic receptor activity. For example, phenylephrine, a specific α_1-adrenergic receptor agonist, is identical in structure to EPI, a mixed α-β-adrenergic receptor agonist, except for the lack of one hydroxyl group on the ring (Table 7.1). Substitution on the β-carbon atom of the side chain produces a compound with reduced central nervous system (CNS) effects, whereas substitution on the α-carbon atom produces a drug that is generally more resistant to oxidation by monoamine oxidase (MAO). The α- and β-agonistic properties of various drugs are affected by alkyl substitutions on the amino group. Collectively, this information supports the idea that pharmacological profiles of specific agonists differ depending upon chemical structure. The chemical structures and related pharmacological characteristics of the endogenous catecholamines and several adrenergic drugs are summarized in Table 7.1.

Activation of Adrenergic Receptors and Cell Signaling

Physiological responses produced by catecholamines and sympathomimetic drugs are initiated by the binding of agonists to adrenergic receptors located on the cell surface (Wilson-Pauwels et al., 1997; Yamada and Ito, 2001; Cazzola et al., 2012; Lei, 2014; Herring, 2015). Adrenergic receptors are G protein-coupled receptors

Table 7.1 Chemical structures of catecholamines and selected sympathomimetic drugs

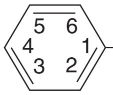
Drug		β CH	α CH	NH
β-phenylethylamine	…	H	H	H
β-phenylethanolamine	…	OH	H	H
Catecholamines				
Dopamine	3-OH, 4-OH	H	H	H
Norepinephrine	3-OH, 4-OH	OH	H	H
Epinephrine	3-OH, 4-OH	OH	H	CH_3
Isoproterenol	3-OH, 4-OH	OH	H	$CH(CH_3)_2$
Noncatecholamines				
Phenylephrine	3-OH	OH	H	CH_3
Tyramine	4-OH	H	H	H
Ephedrine	…	OH	CH_3	CH_3

Table 7.2 Adrenergic receptor types and subtypes, selected agonist and antagonist drugs, primary G proteins involved in mediating cellular responses to activation of specific adrenergic receptors, and typical intracellular effects secondary to adrenergic receptor ligand binding

Receptor	Agonist	Antagonist	G Protein	Effects
α_1-Adrenergic	Phenylephrine	Prazosin	G_q	Activation of PLA_2, PLC, ↑ IP_3 and DAG; common to all
α_{1A}		Tamsulosin		
α_{1B}				
α_{1D}				
α_2-Adrenergic	Dexmedetomidine	Yohimbine Atipamezole	G_i	Inhibition of adenylyl cyclase, ↓ cAMP; common to all
α_{2A}				
α_{2B}				
α_{2C}				
β-Adrenergic	Isoproterenol	Propranolol	G_s	Stimulation of adenylyl cyclase, ↑ cAMP; common to all
β_1	Dobutamine	Atenolol		
β_2	Terbutaline	Butoxamine		
β_3	Mirabegron			
Dopaminergic	Dopamine			
D_1	Fenoldopam		G_s	Stimulation of adenylyl cyclase, ↑ cAMP
D_2	Bromocriptine		G_i	↓ cAMP

PLC, phospholipase C; PLA, phospholipase A; IP_3, inositol-1,4,5-triphosphate; DAG, diacylglycerol; cAMP, cyclic AMP.

(GPCRs) (Calebiro et al., 2010; Jalink and Moolenaar, 2010; Ambrosio et al., 2011; Vischer et al., 2011; Latek et al., 2012; Duc et al., 2015). G proteins are classified by their specific subunits, and select G proteins regulate specific effector proteins and interact with select molecular pathways. Membrane and intracellular processes associated with GPCRs have been previously introduced in Chapter 6.

The β-adrenergic receptor family is composed of three different subtypes (β_1, β_2, and β_3). Activation of β receptors, regardless of subtype, results in stimulation of adenylyl cyclase and increased cAMP, mediated via the stimulatory coupling protein G_s (Table 7.2). β-adrenergic receptors (β_1, β_2, and β_3) are expressed in numerous tissues and organs (Table 7.3), although for a given tissue a specific receptor subtype may be more prominently

Table 7.3 Anatomical distribution of adrenergic receptor subtypes and general physiological effectors produced in response to activation of specific adrenergic receptors

Receptor	Tissue	Response
α_1	Most vascular smooth muscle (innervated)	Contraction
	Pupillary dilator muscle	Contraction (dilates pupil)
	Splenic capsule	Contraction
	Urethral smooth muscle	Contraction
α_2	Postsynaptic CNS neurons	Numerous sites
	Platelets	Aggregation
	Adrenergic and cholinergic nerve terminals	Inhibits transmitter release
	Selected vascular smooth muscle	Contraction
β_1	Heart, juxtaglomerular cells	Increases force and rate of contraction; increases renin release
β_2	Respiratory, uterine, and vascular smooth muscle	Smooth muscle relaxation
	Liver	Gluconeogenesis
β_3	Fat cells	Activates lipolysis
D_1	Vascular smooth muscle	Dilates select blood vessels
D_2	Autonomic nerve terminals	Modulates neurotransmitter release from nerve terminals

expressed when compared with other receptor subtypes, thereby providing the tissue or organ with a relatively specific β-adrenergic subtype signature. For example, cardiac functions, such as increased heart rate and enhanced cardiac contractility, are medicated to a prominent extent by activation of cardiac β_1-receptors, whereas relaxation of bronchial smooth muscle involves activation of bronchial smooth muscle β_2-adrenergic receptors.

β-adrenergic receptors are expressed in smooth muscle at multiple anatomical sites, including: pulmonary airways (β_2 receptors), vascular smooth muscle (β_2 receptors), and the detrusor muscle of the bladder (β_2 and β_3 receptors). Activation of β receptors in these tissues mediates smooth muscle relaxation contributing to bronchodilation, vasodilation of vascular smooth muscle with accompanying reduced vascular resistance, and relaxation of the bladder body. Smooth muscle relaxation secondary to activation of β-adrenergic receptors may be mediated via processes that include the phosphorylation of myosin light-chain kinase to an inactive form. In many species the innervation of vascular smooth muscle β_2-adrenergic receptors by postganglionic sympathetic nerves is rather sparse, suggesting that the primary activation of these receptors is mediated by circulating EPI released from the adrenal medulla. β-adrenergic receptors (generally thought to be β_2 receptors) are also located at presynaptic sites on postganglionic nerve terminals and, in general, activation of presynaptic β_2-adrenergic receptors facilitates the release of NE.

The α_1-receptor family includes three subgroups: $\alpha_{1A,}$ $\alpha_{1B,}$ and $\alpha_{1D.}$ Binding of an agonist to α_1-adrenergic receptors, regardless of subtype, activates phospholipase C, mediated via activation of a G_q coupling protein, which leads in many cases to the formation of inositol 1,4,5-trisphosphate (IP_3) and diacylglycerol, and multiple functional effects including the release of intracellular Ca^{2+} stores (Table 7.2). Although the distribution (Table 7.3) and activation of different α_1-adrenergic receptor subtypes provides the substrate for a diverse profile of physiological responses, the functional signature of α_1-adrenergic receptor activation is smooth muscle contraction, including contraction of vascular smooth muscle, the radial muscle of the iris (iris dilator muscle), and smooth muscle in the genitourinary system. Generally, α_1-adrenergic receptors located in vascular smooth muscle are in close proximity to postganglionic sympathetic nerve endings, therefore they are considered innervated vascular receptors.

The $\alpha_{2\text{-}}$receptor family includes three subgroups: $\alpha_{2A,}$ $\alpha_{2B,}$ and $\alpha_{2C.}$ In many targets the physiological responses to activation of α_2 receptors involves the coupling of these receptors to the inhibitory regulatory protein G_i (Table 7.2), resulting in inhibition of adenylyl cyclase activity and reduced intracellular cAMP levels. It is known that other signaling pathways, separate from the inhibition of adenylyl cyclase, are involved in mediating intracellular processes to $\alpha_{2\text{-}}$receptor activation. α_2-adrenergic receptors are expressed in a variety of cells and tissues (Table 7.3), including vascular smooth muscle, thromobocytes, endothelial cells, and in the CNS. α_2-adrenergic receptors are located on the terminals of postganglionic sympathetic nerve fibers, and activation of these presynaptic adrenergic receptors by endogenous catecholamines and other sympathomimetics reduces NE release from nerve terminals.

By binding to and activating specific receptors at both central and peripheral sites, the endogenous catecholamine dopamine produces numerous physiological effects. Activation of the dopamine$_1$ (D_1) receptor is generally associated with increased cAMP, whereas activation of dopamine$_2$ (D_2) receptors can inhibit adenylyl cyclase activity.

Adrenergic Receptor Selectivity and Tissue Distribution

Overview

The breadth and diversity of (i) adrenergic receptor types and subtypes, (ii) G-proteins, and (iii) second messenger systems provides the cellular and molecular substrate for mediating the myriad number of physiological responses produced by activation of the SNS. In addition, many adrenergic receptor agonists demonstrate a level of selectivity for specific adrenergic receptors. An individual endogenous catecholamine or sympathomimetic drug may exhibit a higher affinity or selectivity for one or more subtypes of adrenergic receptors. Receptor selectivity provides an important framework for understanding target organ effects of endogenous catecholamines and sympathomimetic drugs. However, receptor selectivity for a given agonist is generally not absolute; that is, at higher concentrations a given drug or catecholamine may interact with and activate other subtypes or classes of adrenergic receptors, providing the functional basis for considering a spectrum of relative receptor affinities for most adrenergic agonists. Examples of relative receptor affinities for endogenous catecholamines of select adrenergic drugs are listed in Table 7.4. EPI is an agonist at α_1-, α_2-, β_1-, and β_2-adrenergic receptors, whereas NE is an agonist at α_1-, α_2-, and β_1-adrenergic receptors, with less potent effects on β_2-adrenergic receptors. Isoproterenol is a β_1- and β_2-adrenergic receptor agonist whereas phenylephrine is a selective α_1-adrenergic receptor agonist (Table 7.4).

The anatomical location of specific adrenergic receptor types and subtypes plays a key role in mediating the functional/physiological responses produced by a given

Table 7.4 Classification of adrenergic receptor agonists and their relative selectivity for specific adrenergic receptors

Agonists	Receptor selectivity
Alpha agonists	
Phenylephrine	$\alpha_1 > \alpha_2 >>>>> \beta$
Dexmedetomidine, Medetomidine, Xylazine	$\alpha_2 > \alpha_1 >>>>> \beta$
Mixed alpha and beta agonists	
Norepinephrine	$\alpha_1 = \alpha_2$; $\beta_1 >> \beta_2$
Epinephrine	$\alpha_1 = \alpha_2$; $\beta_1 = \beta_2$
Beta agonists	
Dobutamine	$\beta_1 > \beta_2 >>>> \alpha$
Isoproterenol	$\beta_1 = \beta_2 >>>> \alpha$
Albuterol, Terbutaline	$\beta_2 >> \beta_1 >>>> \alpha$
Dopamine agonists	
Dopamine	$D_1 = D_2 >> \beta >> \alpha$

drug or catecholamine. Pharmacological interactions between specific agonists and adrenergic receptors, as well as the profile of physiological responses produced by agonist–receptor interactions, are diverse and dynamic and are influenced by a number of factors, including: the tissue distribution of adrenergic receptors; the number of adrenergic receptors expressed at specific sites; interactions between the sympathetic and parasympathetic nervous systems at target sites; specific pathophysiological and disease states; the background level of sympathetic nerve activity; and levels of endogenous catecholamines and/or sympathomimetic drugs. Regarding the latter, prolonged exposure of adrenergic receptors to specific agonists reduces the responsiveness of these receptors to agonist activation, producing a progressive attenuation in the tissue's capacity to facilitate physiological responses. This physiological phenomenon is known as desensitization of adrenergic receptors. Multiple cellular and molecular mechanisms likely play a role in mediating adrenergic receptor desensitization, including changes in the number of adrenergic receptors and in intracellular signaling mechanisms.

Cardiovascular Responses to Adrenergic Receptor Agonists: Effects of Adrenergic Receptor Selectivity and Distribution

The pivotal contributions of adrenergic receptor selectivity and tissue distribution in mediating physiological responses to sympathomimetic drugs can be highlighted by comparing the effects of specific endogenous catecholamines and sympathomimetic drugs on various physiological regulatory systems, including cardiovascular regulation. In this section the cardiovascular effects (blood pressure, heart rate, myocardial contractility, cardiac output) produced by the administration of phenylephrine (selective α_1-adrenergic receptor agonist), NE and EPI (endogenous catecholamines), and isoproterenol (β_1- and β_2-adrenergic receptor agonist) are compared, with an emphasis on integrating and explaining how differences in their affinity or selectivity for specific α- and β-adrenergic receptors, and the primary anatomical location of specific adrenergic receptors, contribute to the

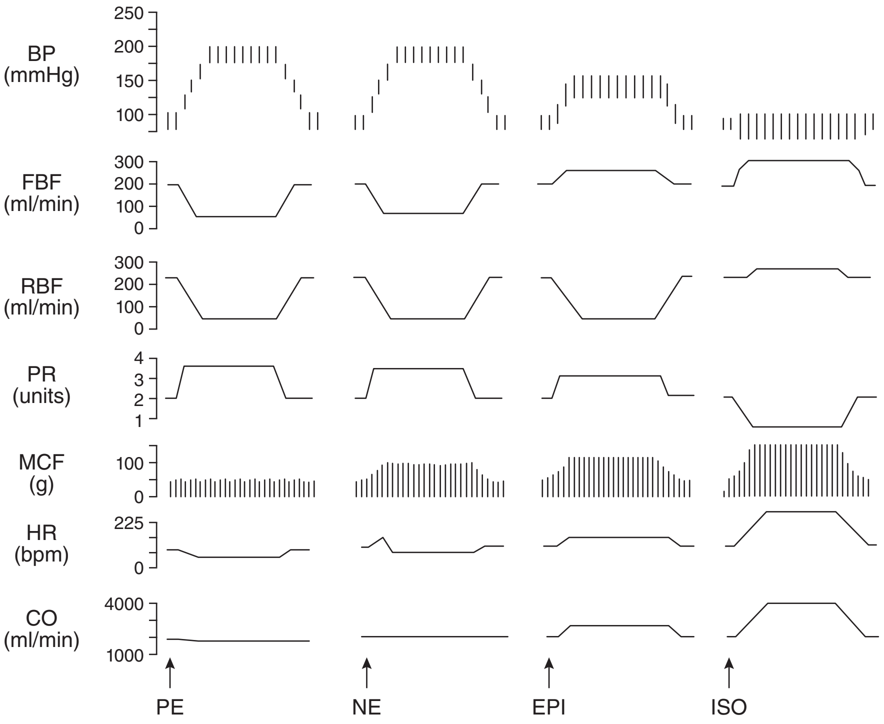


Figure 7.2 Schematic showing cardiovascular effects in response to the intravenous administration of four adrenergic agonists (phenylephrine (PE), norepinephrine (NE), epinephrine (EPI), and isoproterenol (ISO)) characterized by different relative receptor affinity profiles. Schematic representations of approximate equivalent doses of these endogenous catecholamines and sympathomimetic amines on blood pressure (BP), femoral blood flow (FBF), renal blood flow (RBF), peripheral vascular resistance (PR), myocardial contractile force (MCF), heart rate (HR), and cardiac output (CO). See text for explanation of the cardiovascular responses.

Table 7.5 Selected adrenergic receptors that play a role in cardiovascular regulation, their primary distributions in the vasculature and heart, general cardiovascular responses produced by their activation, and the selectivity of agonists (EPI, NE, ISO, and PE) for the specific adrenergic receptors

Receptor	Tissue	Physiological response	Agonist selectivity
α	Blood vessels	Vasoconstriction	PE; EPI > NE >>> ISO
β_1	Heart	Augmented inotropic and chronotropic effects	ISO > EPI $\geq$ NE
β_2	Blood vessels	Vasodilation	ISO > EPI >>> NE

EPI, epinephrine; ISO, isoproterenol; NE, norepinephrine; PE, phenylephrine.

observed cardiovascular responses (Figure 7.2). Arterial blood pressure is determined by the flow of blood through a vessel, which is ultimately determined by cardiac output and by the diameter or tone of that vessel, represented as peripheral vascular resistance. Information regarding the specific adrenergic receptors that play a role in cardiovascular regulation, their primary anatomical distributions in the vasculature and heart, the specific cardiovascular responses produced by their activation, and the order of potency of EPI, NE, isoproterenol, and phenylephrine are summarized in Table 7.5. This information provides the background required for explaining differences in cardiovascular response profiles to specific adrenergic agonists (Figure 7.2). In the examples described in this section each of the adrenergic receptor agonists have been administered as bolus injections over a short period of time.

Phenylephrine is a potent and selective agonist at α_1-adrenergic receptors. α_1-receptors are expressed in numerous vascular beds (e.g., visceral organs, skin, and skeletal muscle) and their activation by phenylephrine administration produces vasoconstriction of arterial smooth muscle, which in turn increases peripheral vascular resistance and reduces femoral blood flow and renal blood flow (Figure 7.2). In addition, phenylephrine administration produces venoconstriction, which reduces venous capacitance and increases venous return. The increase in arterial blood pressure is primarily due to the marked α_1-mediated increase in peripheral vascular resistance. The abrupt increase in arterial blood pressure secondary to phenylephrine administration activates the arterial baroreflex, which elicits a reflex-mediated increase in cardiac vagal nerve activity and a reduction in heart rate (bradycardia). α_1-receptors are not prominently expressed on cardiac cells involved in the regulation of cardiac rate or myocardial contractility; therefore phenylephrine administration does not elicit substantial direct effects on heart rate or cardiac output. However, from an integrative perspective phenylephrine administration does affect several of the physiological indices involved in the regulation of cardiac output, including: increased peripheral vascular resistance and afterload; increased venous return to the heart; and reduced heart rate via activation of the baroreflex.

Norepinephrine is an agonist at α-adrenergic (α_1 and α_2) and β_1-adrenergic receptors (and to a much lesser extent to β_2-adrenergic receptors). Activation of vascular α_1-adrenergic receptors by NE increases peripheral vascular resistance, as evidenced by decreases in femoral and renal blood flow (Figure 7.2). In addition, NE administration produces venoconstriction, which reduces venous capacitance and increases venous return. The activation of α_2-adrenergic receptors in vascular smooth muscle produces vasoconstriction and likely contributes to NE-induced increases in peripheral vascular resistance. β_1-adrenergic receptors are expressed in the heart, and activation of cardiac β_1-receptors by NE produces positive inotropic (increased cardiac contractility) and chronotropic (increased rate) effects. The increase in arterial blood pressure is mediated by combined vascular (α-adrenergic receptor-mediated vasoconstriction) and cardiac (increased cardiac output due to activation of β_1-adrenergic receptor) effects. The marked NE-induced increase in arterial blood pressure activates the arterial baroreflex, which elicits a reflex-mediated increase in cardiac vagal nerve activity and a reduction in heart rate. The reflex bradycardia does not necessarily lead to a reduction in cardiac output because of the direct effect of NE to activate cardiac β_1-receptors and increase myocardial contractility and stroke volume.

Epinephrine is a potent agonist at α-adrenergic (α_1 and α_2) and β-adrenergic (β_1 and β_2) receptors, and activation of vascular β_2-adrenergic receptors produces vasodilation. Therefore, EPI administration produces vasoconstriction and reduces blood flow in vascular beds that contain high concentrations of α-adrenergic receptors (e.g., visceral organs, cutaneous vessels), accounting for the EPI-induced reduction in renal blood flow (Figure 7.2). Some targets, such as the smooth muscle in the arterial vasculature supplying skeletal muscle, contain both β_2- and α-adrenergic receptors, and EPI-induced activation of β_2 receptors produces vascular vasodilation, whereas EPI-induced activation of α-adrenergic receptors produces vasoconstriction. In general, skeletal muscle vascular beds are often characterized by reduced peripheral vascular resistance in response to EPI administration (demonstrated as increased femoral blood flow in Figure 7.2), whereas EPI produces α-adrenergic receptor-mediated vasoconstriction in visceral vascular beds. Due to the competing vasoconstrictor and vasodilatory effects of EPI, the increase in peripheral vascular resistance to EPI administration is generally not as robust as that observed in response to NE administration (Figure 7.2). EPI is a prominent cardiac stimulant and activation of cardiac β_1-adrenergic receptors increases cardiac contractility, heart rate, and cardiac output. The initial effect of EPI administration is often characterized by a rapid increase in cardiac output (cardiac β_1-receptor activation), a marked increase in peripheral vascular resistance mediated by the activation of visceral α-adrenergic receptors, and an increase in arterial blood pressure, which in turn may elicit a baroreflex-mediated increase in cardiac vagal nerve activity and a transient reduction in heart rate. Subsequent activation of β_2-adrenergic receptors as EPI is distributed to peripheral sites, such as skeletal muscle vascular beds, attenuates increases in peripheral vascular resistance and arterial blood pressure.

Isoproterenol is a potent, nonselective β_1- and β_2-adrenergic receptor agonist that exerts little effect on α-adrenergic receptors; therefore blood pressure responses to this β-adrenoceptor agonist are a function of its effects on cardiac and peripheral vascular β-adrenergic receptors. The vascular response profile to isoproterenol administration is characterized by vascular vasodilation, an increase in blood flow to vascular beds that contain a high density of β_2-adrenergic receptors (skeletal muscle, as supported by the increase in femoral blood flow), modest β_2-adrenergic receptor-mediated vasodilation in visceral vascular beds (e.g., renal blood flow), and reduced peripheral vascular resistance (Figure 7.2). Because isoproterenol lacks affinity for α-adrenergic receptors, there is no pharmacological-induced vascular smooth muscle contraction; therefore isoproterenol produces a marked reduction in peripheral vascular resistance. Isoproterenol is a potent cardiac stimulant. Isoproterenol activation of cardiac β_1-adrenergic receptors increases cardiac output by increasing heart rate, mediated by direct activation of the sinus node, and by increasing contractility of myocardial cells. It is generally considered that β_1-adrenergic receptors are the predominate type of myocardial β-adrenergic receptor. Mean arterial blood pressure is typically reduced in response to isoproterenol secondary to the marked reduction in peripheral vascular resistance, despite the accompanying increase in cardiac output.

Comparing cardiovascular responses to specific catecholamines and select sympathomimetic drugs supports the concept that relative receptor affinity, direct receptor effects, receptor distribution, and intrinsic reflex adjustments are key components for predicting drug-associated physiological response profiles for a variety of organs and regulatory systems.

Clinical Applications of Adrenergic Receptor Agonists

As stated previously, adrenergic receptors are distributed widely throughout many tissues and organ systems, and activation of these receptors facilitates a myriad of physiological responses. Selected clinical applications of sympathomimetics in specific conditions are described in this section as a framework for characterizing the pharmacodynamic effects of these drugs.

Adrenoceptor Agonists and Respiratory Physiology

A 15-year-old Quarter Horse mare presented with the clinical complaint of cough and increased abdominal effort during the expiratory phase of respiration. Physical examination revealed a heart rate of 44 beats per minute (normal; 36–44 beats per minute) and a respiratory rate of 20 breaths per minute (normal; 12–24 breaths per minute). Thoracic auscultation revealed increased bronchovesicular sounds and expiratory wheezes in all lung fields. In addition to an increased respiratory effort, the mare demonstrated prominent nostril flare (wide open nostrils) with expiration. Arterial blood gas analysis indicated hypoxemia as demonstrated by an arterial partial pressure of oxygen ($Pa0_2$) of 66 mmHg.

Based on the clinical findings, an immediate treatment protocol included the administration of aerosolized albuterol (selective β_2-adrenergic receptor agonist) (Rush et al., 1999). β_2-adrenergic receptors are present in bronchial smooth muscle, and activation of these receptors by β_2-agonists results in relaxation of bronchial smooth muscle and bronchodilation (Boushey, 2007; Cunningham, 2007). Approximately 10 minutes after albuterol administration, thoracic auscultation had improved substantially. Continued therapy included corticosteroid with albuterol, which was maintained over the course of 72 hours and the arterial partial pressure of oxygen level ($Pa0_2$) improved to 97 mmHg (Cornelisse et al., 2004; Boushy, 2007). In addition to improved oxygen delivery, the mare exhibited an improved respiratory effort demonstrated by resolution of her expiratory effort and nasal flare. The owner indicated the horse lived in a small pen with a round bale of hay that was stored outdoors.

This patient is suffering from recurrent airway obstruction, an allergic form of airway disease (Cunningham, 2007; Ainsworth and Cheetham, 2010). The condition is similar to asthma in humans. Airway obstruction results from hypersensitivity reaction to molds located in the forage. Following inhalation of molds, airway inflammation is induced in the form of neutrophil inflammation with marked mucous accumulation and airway smooth muscle contraction (Ainsworth and Cheetham,

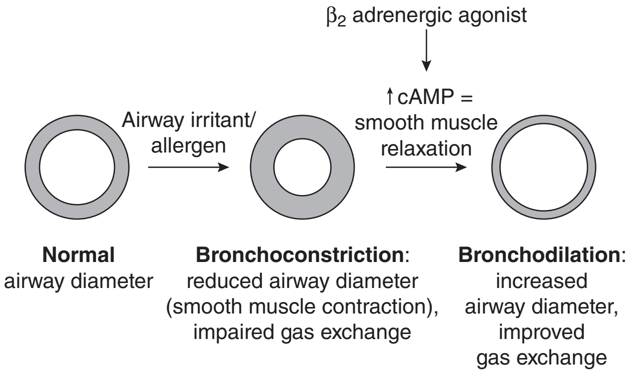

β_2 adrenergic agonist
↑cAMP = smooth muscle relaxation
Airway irritant/ allergen
Normal
airway diameter
Bronchoconstriction:
reduced airway diameter (smooth muscle contraction), impaired gas exchange
Bronchodilation:
increased airway diameter, improved gas exchange

Figure 7.3 Airway irritants produce bronchoconstriction mediated via activation of inflammatory cascades and the parasympathetic nervous system (PSNS). The PSNS is responsible for maintaining baseline airway tone and activation of bronchial muscarinic receptors produces contraction of bronchial smooth muscle. Administration of a β_2-adrenergic receptor agonist and subsequent activation of bronchial smooth muscle β_2-adrenergic receptors increases intracellular cAMP levels with subsequent activation of cAMP-dependent protein kinase A, resulting in relaxation of bronchial smooth muscle.

2010). Therapeutic goals for affected individuals involve environmental management to reduce mold exposure and bronchodilation through the use of β_2-adrenergic receptor therapy. Corticosteroid therapy is aimed at reducing the hypersensitivity reaction (neutrophilic) to aerosolized allergens (Boushey, 2007; Ainsworth and Cheetham, 2010).

Control of bronchial smooth muscle tone depends on the integration of signals from several types of receptors that respond to chemical, mechanical, and physical stimuli (Cunningham, 2007; Guyton and Hall, 2011). The parasympathetic nervous system is responsible for maintaining baseline airway tone and activation of bronchial muscarinic receptors produces contraction of bronchial smooth muscle. On the other hand, activation of bronchial smooth muscle β_2-adrenergic receptors increases intracellular cAMP levels with subsequent activation of cAMP-dependent protein kinase A, which results in relaxation of bronchial smooth muscle (Figure 7.3).

Epinephrine and isoproterenol are potent bronchodilators, whereas NE exerts less of an effect due to its limited affinity for β_2-adrenergic receptors (Boushey, 2007). Several selective β_2-adrenergic receptor agonists are available for use in veterinary patients. Terbutaline is commonly used for small animal patients, can be administered parenterally or orally, and is useful for the treatment of airway constriction in dogs and cats (Boushey, 2007). In equine patients albuterol can be administered as a short-acting (1–2 hour) aerosolized bronchodilator for conditions that involve airway obstruction (bronchoconstriction) (Rush et al., 1999). Clenbuterol is an alternate bronchodilator prepared for oral administration as a syrup. A significant advantage of clenbuterol is a longer duration of action (12 hours). Clenbuterol is FDA approved for use in horses with airway constriction. It should be noted that clenbuterol is illegal for use in food-producing animals (Boushey, 2007; Ainsworth and Cheetham, 2010).

Sympathomimetics can be administered parenterally or by aerosol formulation to achieve rapid and effective bronchodilation for the treatment of allergic reactions or status asthmaticus. The selective β_2-adrenergic receptor agonists do not elicit the same number of additional physiological responses as do EPI and isoproterenol (Boushey, 2007; Guyton and Hall, 2011).

Arterial Blood Pressure Regulation and Sympathomimetics

A male Labrador retriever presented with a cranial cruciate rupture and was prepared for surgery. The patient was premedicated with hydromorphone, anesthesia was induced with propofol, and was maintained with isoflurane (volatile anesthetic) in oxygen. During the 20 minutes after initiation of isoflurane administration, systolic blood pressure gradually decreased from an initial level of 100 mmHg to 75 mmHg. The depth of anesthesia was determined to be appropriate, the heart rate was 92 beats/minute and the patient was normovolemic. What pharmacological interventions might be appropriate to treat the arterial hypotension in this patient?

When choosing a drug to treat hypotension, potential causes of the reduction in arterial blood pressure need to be considered. Isoflurane is a potent vasodilator, even at subanesthetic doses, and a reduction in peripheral vascular resistance is the predominant mechanism for the decrease in blood pressure. In addition, although cardiac output is preserved at low-dose isoflurane, the maintenance of surgical anesthesia for the type of surgery described for this condition may require higher doses of isoflurane, which are known to reduce cardiac output secondary to direct depressant effects on myocardial contractility. Under the conditions of this clinical case the isoflurane-induced hypotension would most effectively be ameliorated by a drug that can mediate increases in systemic vascular resistance and cardiac output; therefore, sympathomimetics that activate both α_1- and β_1-adrenergic receptors would be considered.

The drugs most frequently selected for treatment of hypotension in veterinary medicine under the described clinical situation would include dopamine, ephedrine, and dobutamine. Dopamine is an agonist at DA_1 receptors and the intravenous administration of low-dose dopamine (approximately, 0.5 to 2 μg/kg/min) promotes vasodilation of numerous vascular beds, including the renal and splanchnic vasculatures, an effect that would not be consistent with the express purpose of reversing isoflurane-induced hypotension. However, dopamine demonstrates complex, dose-dependent adrenergic receptor agonist properties. At moderate infusion rates (2 to 10 μg/kg/min) dopamine activates cardiac β_1-receptors, leading to increases in cardiac contractility, heart rate, and cardiac output. At higher rates of infusion (10 to 20 μg/kg/min), dopamine activates vascular α_1-adrenergic receptors, leading to vasoconstriction and increased peripheral vascular resistance. At the higher dose, dopamine remains an agonist at cardiac β_1-adrenergic receptors; therefore, high-dose dopamine infusion increases peripheral vascular resistance and enhances cardiac contractility, positioning dopamine as a suitable choice for isoflurane-induced hypotension. Dopamine has a short half-life and is given by a constant-rate intravenous infusion.

Ephedrine is a noncatecholamine drug that is an agonist at α_1-, β_1-, and β_2-adrenergic receptors, and can also induce the release of NE from postganglionic sympathetic nerve terminals. The cardiovascular effects of ephedrine have been shown to be dose dependent in isoflurane-anesthetized dogs (Wagner et al., 1993). Low-dose ephedrine (0.1 mg/kg) produces transient yet substantial increases in mean arterial pressure, cardiac index, and stroke volume, as well as decreases in heart rate and systemic vascular resistance. These data suggest that low-dose ephedrine may activate primarily cardiac β_1- and vascular β_2-adrenergic receptors. At a higher dose (0.25 mg/kg) ephedrine induces more marked and sustained increases in mean arterial pressure, cardiac index, and stroke volume, and also mediates an increase in systemic vascular resistance, suggesting that higher doses of ephedrine likely activates vascular α_1-adrenergic receptors. The combined cardiovascular effects produced by high-dose ephedrine make this drug an effective treatment option for isoflurane-induced hypotension.

Although at clinical doses dobutamine can act as an agonist at β_2- and α_1-adrenergic receptors, for the most part this drug demonstrates a relative selectivity for β_1-adrenergic receptors, especially those subserving changes in myocardial contractility (rather than cardiac rate). Thus, dobutamine administration increases myocardial contractility, cardiac index, and stroke volume. In this regard, dobutamine is particularly useful in treating low cardiac output flow states such as congestive heart failure or dilated cardiomyopathy. However, the rather modest agonistic properties at α_1-adrenergic receptors, at least in dogs, often make the use of dobutamine a less effective treatment option for isoflurane-induced hypotension (Rosati et al., 2007).

Epinephrine, NE, and phenylephrine are additional sympathomimetics that can increase arterial blood pressure, although they are not generally used under the conditions of isoflurane-induced hypotension. However, EPI is effective in life-threatening circumstances such as asystole or anaphylaxis. EPI is a potent cardiac stimulant and vasoconstrictive agent and can reverse the marked hypotension and cardiac irregularities associated with anaphylactic shock. Blood pressure responses to a given infusion rate of EPI are often associated with a substantial degree of interpatient variability; therefore, arterial blood pressure must be carefully monitored with EPI administration. EPI is a potent renal vasoconstrictor and can result in significant decreases in renal blood flow at higher doses. Additionally, some inhalation anesthetics, predominately halothane, can sensitize the heart to catecholamines. EPI decreases the refractory period and the heart is more susceptible to ventricular arrhythmias.

Because of its potent α_1-adrenergic receptor agonistic activity, NE is used clinically in hypotensive situations. NE administration induces marked constriction of arteries and veins and is used to support vascular resistance in circumstances such as vascular collapse secondary to sepsis. Like EPI, it is a potent constrictor of renal and mesenteric vascular beds, which may ultimately lead to decreased perfusion of those organ systems. Administration of selective α_1-adrenergic agonists, such as phenylephrine, produce peripheral vasoconstriction and are used in circumstances where cardiac output is adequate such as the hypotension resulting from spinal or epidural local anesthesia and subsequent block of sympathetic nerves.

Adrenergic Drugs and Urinary Incontinence

Adrenergic receptors are expressed at numerous sites in the urinary tract including the ureters (β_2 receptors), detrusor muscle of the bladder body (β_2 and β_3 receptors), bladder base (α_1 receptors), and the internal urethral sphincter (α_1 receptors). Stimulation of sympathetic nerves innervating these sites or activation of these receptors secondary to administration of sympathomimetics produces smooth muscle relaxation of the bladder body via β_2-adrenergic receptors (and β_3 receptors), and smooth muscle contraction at the bladder base and the internal urethral sphincter by activation of α_1-adrenergic receptors. The latter mechanism is the basis for treatment of urinary incontinence in veterinary patients using the α_1-adrenergic receptor agonists.

Urethral sphincter incompetence is the most common cause of acquired urinary incontinence in female dogs and cats. A single daily dose of phenylpropanolamine results in an increase in urethral pressure values and improved urinary continence in most affected dogs (Claeys et al., 2001). As expected, phenylpropanolamine administration can produce significant increases in arterial blood pressure, mediated by activation of vascular smooth muscle, and a baroreflex-mediated compensatory decrease in heart rate (Carofiglio et al., 2006). The consequences of phenylpropanolamine-induced increases in systemic vascular resistance and afterload should be considered when prescribing this drug for urinary incontinence.

Clinical Applications of Sympathomimetic-Induced Vasoconstriction

Drugs that are selective α-adrenergic agonists, or have potent α-adrenergic activity and minimal β_2-adrenergic effects, can cause significant vasoconstriction and are used for a number of clinical applications. These include the addition of EPI to local anesthetics to delay removal of the anesthetic from the site of injection, thereby prolonging its effect; the reduction of local perfusion to affect hemostasis in areas such as the nose or mouth; and the treatment of hypotension secondary to marked vasodilation but in the presence of normal or increased cardiac output, a response profile that occurs after sympathetic nerve blockade with spinal or epidural anesthesia.

α_1-adrenergic receptors are expressed on the smooth muscle of the splenic capsule and SNS activation to this target produces smooth muscle contraction, as does administration of sympathomimetics such as phenylephrine. Splenic smooth muscle contraction discharges red blood cells into the circulation and reduces the size of the spleen. Reduction of splenic size produced in response to the intravenous administration of the α_1-adrenergic selective receptor agonist phenylephrine is often used as part of the therapy for treatment of nephrosplenic entrapment in horses. This condition occurs when the left ventral and dorsal colon migrate between the spleen and the body wall and become entrapped over the nephrosplenic ligament, resulting in an obstruction of the large colon (Hardy et al., 2000). Phenylephrine administration reduces splenic area and thickness, and may increase the success of nonsurgical correction (Hardy et al., 1994). As expected, phenylephrine infusion increases peripheral vascular resistance and arterial blood pressure, and produces a marked baroreflex-mediated reduction in heart rate. The use of phenylephrine as a treatment option for nephrosplenic entrapment has been implicated in producing life-threatening internal hemorrhage in aged horses, and the use of this sympathomimetic as a treatment modality in older horses should be carefully considered (Frederick et al., 2010).

Peripheral and Central Sites Mediate Cardiovascular Responses to α_2-Receptor Activation

Circulating catecholamines do not readily cross the blood–brain barrier; however, certain sympathomimetic agents can gain access to the CNS and activate adrenergic receptors found in the brain and spinal cord. α_2-adrenergic receptor agonists (e.g., xylazine, detomidine, medetomidine, and dexmedetomidine) are routinely used in veterinary patients for sedation and analgesia. The sedative and analgesic effects of these agents are consistent with activation of central neural receptors and pathways. Accompanying the clinically desired sedative and analgesic effects are pronounced cardiovascular and sympathetic neural changes in response to administration of α_2-adrenergic selective receptor agonists (Kenney et al., 2014). The cardiovascular responses provide a window for characterizing physiological responses produced by activation of peripheral and central α_2-adrenergic receptor subtypes.

The α_2-adrenergic receptor family includes three subgroups: $\alpha_{2A,}$ $\alpha_{2B,}$ and $\alpha_{2C.}$ α_2-adrenergic receptors are present in vascular smooth muscle, on the terminals of postganglionic sympathetic nerve fibers, and in the central nervous system, including in the brainstem. The intravenous administration of selective α_2-adrenergic receptor agonists produce a biphasic cardiovascular profile, an initial transient hypertension followed by a longer lasting hypotension. The following description of the biphasic cardiovascular response is based on the intravenous administration of dexmedetomidine, and is representative of responses induced by α_2-adrenergic selective receptor agonists.

Activation of α_2-adrenergic receptors by intravenous dexmedetomidine administration produces vasoconstriction of arterial smooth muscle, which increases peripheral vascular resistance and produces an immediate increase in arterial blood pressure. It is thought that the dexmedetomidine-induced contraction of vascular smooth muscle is mediated by activation of α_{2B}-adrenergic receptors. The abrupt increase in arterial blood pressure activates the arterial baroreflex, which elicits a reflex-mediated increase in cardiac vagal nerve activity and a reduction in heart rate. Circulating dexmedetomidine rapidly gains access to CNS α_2-adrenergic receptors and activation of brainstem receptors produce inhibition of sympathetic nerve outflow and activation of parasympathetic nerve outflow. It is thought that the central neural effects of dexmedetomidine on sympathetic and parasympathetic neural circuits are mediated by activation of α_{2A}-adrenergic receptors. The dexmedetomidine-induced hypotensive response is mediated by the sustained reduction in sympathetic nerve outflow, as well as the sustained increase in parasympathetic nerve outflow, which mediates a reduction in heart rate.

Ophthalmology and Adrenergic Agonists

A 9-year-old golden retriever presented with cloudy lenses and the owner reported the animal had decreased vision. The ophthalmic examination revealed bilateral advanced immature cataracts. To facilitate intraoperative mydriasis (pupillary dilation), the selective α_1-adrenergic receptor agonist, phenylephrine, is used in dilation protocols for dogs prior to cataract surgery. α_1-adrenergic receptors are expressed on the radial dilator muscle of the iris (iris dilator muscle) and on conjunctival blood vessels. SNS activation to this target causes mydriasis, as does α_1-adrenergic receptor activation by sympathomimetics such as phenylephrine. Following topical application, phenylephrine produces contraction of the smooth muscle of the conjunctival blood vessels and contraction of the iris dilator muscle, causing blanching of the conjunctival vasculature and pupillary dilation, respectively. Topical phenylephrine should be used with caution in small patients due to the potential for phenylephrine to enter the systemic circulation and induce arterial hypertension secondary to activation of peripheral vascular α_1-adrenergic receptors (Pascoe et al., 1994). Phenylephrine is commercially available in 2.5% and 10% solutions.

A dilute solution of phenylephrine may also be used in the neuroanatomic localization of Horner's syndrome, an oculosympathetic palsy (Webb and Cullen, 2013). After application of a dilute solution of phenylephrine to both eyes in unilateral postganglionic Horner's syndrome, the affected pupil should dilate within 20 minutes and other clinical signs (ptosis, enophthalmia, and third eyelid elevation) will improve or completely resolve.

Apraclonidine and brimonidine are α_2-agonists that have intraocular pressure lowering effects in humans, secondary to increasing the outflow of aqueous humor from the eye. These medications are not recommended for veterinary patients due to systemic side effects and lack of significant intraocular pressure lowering effects (Miller and Rhaesa, 1996; Gelatt and MacKay, 2002).

Use of β-Adrenergic Agonists in Food Production

Beta-adrenergic receptor agonists (βAA) have been used as growth promotion agents for livestock in the United States since 1999 when ractopamine hydrochloride was approved in swine (FDA, 1999). Since that time, ractopamine has been approved for use in cattle and turkeys and a second βAA, zilpaterol, was approved for use in cattle in the United States, Mexico, Canada, and South Africa (FDA, 2003; FDA, 2006). Ractopamine is primarily a β_1-adrenergic receptor agonist with some β_2-receptor activity. Zilpaterol is primarily a β_2-adrenergic receptor agonist. It is also important to note that not all βAA are approved for use in food animals. In the United States, it is illegal to feed a βAA or any other drug in a manner that is inconsistent with its labeling as approved by the FDA. Extra-label drug use is strictly prohibited. These drugs are fed at the end of the animals feeding period only. The use of these drugs in food animals is further discussed in Chapter 52.

The muscle growth in response to βAA treatment appears to be a true muscle hypertrophy, such that muscle fibers increase in diameter due to increased protein synthesis and decreased protein degradation without the incorporation of additional DNA from satellite cells (Yang and McElligott, 1989). However, with longer duration of βAA administration the rate of hypertrophy cannot be maintained without additional DNA and the responsiveness to the βAA is dampened and the accretion of skeletal muscle decreases. The response of muscle cells to βAA stimulation is highly dependent on receptor presence, which is influenced by animal maturity and receptor density, which can be reduced with chronic βAA administration. Additionally, the muscle hypertrophy within the carcass is not evenly distributed, as skeletal muscles that are used for locomotion, which typically have an increased blood supply, have a greater degree of hypertrophy compared to apaxial muscles (Hilton et al., 2010). Muscle fiber types are also differentially affected by β_2AA administration as the increase in muscle fiber diameter is greater in type IIA fibers compared with type I fibers. Beta receptor specificity of skeletal muscle varies between species used for food production. Ruminants tend to have an increased number of β_2-adrenergic receptors than other species. The distribution of subtypes of receptors also varies with the age of the animal, with a more pronounced response to β_2AA in yearling cattle, when compared to calves or fetal responses (Beermann, 2002; Johnson et al., 2014).

Glycogenolysis is increased by βAA stimulation of muscle (Etherton, 1994). Glycogenolytic effects are induced by the phosphorylation of glycogen phosphorylase, which converts glycogen, an intracellular stored form of glucose, to monomeric glucose-6-phosphate. Glucose-6-phosphate is a substrate source for glycolysis, or anaerobic respiration, resulting in the production of ATP and pyruvate. Pyruvate will then enter the Krebs cycle and undergo aerobic respiration, assuming that enough cellular oxygen is available. If the cell is in a hypoxic state, pyruvate will be converted to lactate by lactate dehydrogenase. Lactate will then accumulate and be removed from the cell via blood circulation. The end result of βAA-mediated glycogenolysis is to make an energy source stored within the muscle cell rapidly available.

In adipose cells, βAA promote lipolysis associated with stimulating triacylglycerol hydrolysis and inhibiting lipogenesis stimulated through the β-receptor–G protein–cAMP–PKA pathway (Lafontan et al., 1988; Bergen, 2001; Johnson et al., 2014). The βAA-induced increase in cAMP results in the phosphorylation of acetyl-CoA carboxylase inhibiting the de novo biosynthesis of fatty acids. The net response of adipose tissue to βAA is lipolytic and causes a release of fatty acids, which may be used as an energy source by conversion to acetyl-CoA (Blum and Flueckiger, 1988; Johnson et al., 2014). High concentrations of acetyl-CoA can inhibit conversion of pyruvate to acetyl-CoA, forcing pyruvate to be metabolized to lactate by lactate dehydrogenase and potentially increasing metabolic acidosis in cases where lactic acidosis is present, such as hypoxic situations (Haffner and Kendall, 1992).

β-adrenergic receptor agonists exert significant cardiovascular effects as described earlier in this section. The use of ractopamine and zilpaterol has been shown to increase heart rate in cattle, with the most significant increase at the beginning of the feeding period.

Adrenergic-Receptor Antagonists

The physiological effects produced by activation of the SNS or the administration of sympathomimetic drugs can be diminished by blocking adrenergic receptors (adrenoceptor antagonists), decreasing the amount of NE released presynaptically, or suppressing sympathetic outflow from central sympathetic neural circuits. Drugs that inhibit the interaction of NE, EPI, and other sympathomimetics with α- and β-adrenergic receptors are termed adrenergic receptor antagonists or adrenoceptor antagonists.

Adrenergic receptor antagonists demonstrate selectivity and specificity for the various adrenergic receptors, and drugs are classified based on their antagonism of α- or β-adrenergic receptors. Antagonism of peripheral dopaminergic receptors is of little clinical relevance in veterinary medicine at the present time. Blockade of dopaminergic receptors in the CNS have significant clinical relevance and these drugs are discussed in Chapter 9. The relative selectivity for adrenergic receptor antagonists are presented in Table 7.6.

Table 7.6 Classification of adrenergic receptor antagonists and their relative selectivity for specific adrenergic receptors

Drugs	Receptor selectivity
Alpha antagonists	
Prazosin, Terazosin, Doxazosin	$\alpha_1 >>>> \alpha_2$
Phenoxybenzamine	$\alpha_1 > \alpha_2$
Phentolamine	$\alpha_1 = \alpha_2$
Atipamezole, Yohimbine, Tolazoline	$\alpha_2 >> \alpha_1$
Mixed antagonists	
Carvedilol	$\beta_1 = \beta_2 \geq \alpha_1 > \alpha_2$
Beta antagonists	
Propranolol, Timolol	$\beta_1 = \beta_2$
Metoprolol, Atenolol, Esmolol, Betaxolol	$\beta_1 >>> \beta_2$

Alpha-Adrenergic Receptor Antagonists

Alpha-adrenoceptor antagonists are a chemically heterogeneous and structurally diverse group of drugs. Some of the more clinically relevant groups include β-haloethylamine alkylating agents (e.g., phenoxybenzamine), imidazoline analogs (e.g., phentolamine and tolazoline), piperazinyl quinazolines (e.g., prazosin), and indole derivatives. These drugs are predominantly competitive antagonists at α-adrenergic receptors, with the exception of phenoxybenzamine which irreversibly binds to the α-adrenergic receptor. The relative receptor affinity of individual α_1- and α_2-receptor antagonists varies markedly, and recent research advances have developed drugs that discriminate among the receptor subtypes. Additionally, the phenothiazine tranquilizers (e.g., acepromazine) demonstrate substantial α-adrenergic receptor blocking effects, but since this is generally considered a side effect of these drugs, as opposed to an indication for their use, these drugs will not be discussed in this chapter.

Cardiovascular Effects

Some of the most significant clinical and therapeutic effects, particularly of the α_1-antagonists, target the cardiovascular system. Systemic administration of α_1-adrenergic receptor antagonists produces dilation of both arterial and venous vessels, resulting in reduced blood pressure secondary to decreases in peripheral vascular resistance. As expected, administration of α_1-adrenergic receptor antagonists block or eliminate the vasoconstriction and blood pressure responses produced by exogenous administration of sympathomimetic drugs or neurotransmitters. For example, increases in peripheral vascular resistance and subsequent elevations in blood pressure produced by phenylephrine (selective α_1-adrenergic receptor agonist) administration are completely eliminated by pretreatment with an α_1-adrenergic antagonist. Following pretreatment with an α_1-adrenergic receptor antagonist, increases in peripheral vascular resistance to NE administration are eliminated, which markedly attenuates NE-induced increases in arterial blood pressure despite its effect to activate cardiac β_1-adrenergic receptors and increase heart rate and cardiac contractility. Furthermore, following pretreatment with an α_1-adrenergic receptor antagonist, administration of EPI may induce a reduction in peripheral vascular resistance and marked vasodilation mediated by activation of β_2-adrenergic receptors, resulting in decreased levels of arterial blood pressure. This effect is known as EPI reversal and can occur not only with exogenous administration of the sympathomimetic following administration of an α_1-adrenergic receptor antagonist, but may also occur when administering an α_1-adrenergic receptor antagonist to a patient with high circulating levels of EPI.

Noncardiovascular Effects

Activation of sympathetic nerves innervating the urethra produces contraction of urethral smooth muscle by activation of α_1-adrenergic receptors. α_1-adrenergic receptor antagonists decrease resistance to urine flow. α_{2B}-adrenergic receptors have a role in platelet aggregation; however, the clinical significance of blocking these receptors and the role it might play in antiaggregant therapy is not well understood. α_2-adrenergic receptors inhibit insulin secretion from pancreatic islet cells and antagonism of these receptors may stimulate insulin release. Other effects produced by α-adrenergic receptor antagonists include protrusion of the third eyelid, miosis, and nasal stuffiness. α_2-adrenergic receptor agonists are frequently used to provide sedation and analgesia to veterinary patients, and there are several α_2-adrenergic receptor antagonists, that can reverse the sedation mediated by the α_2-adrenergic receptor agonists. Specific physiological effects of available α_2-adrenergic receptor antagonists will be discussed under the section on specific agents.

Specific Agents and Clinical Uses

Nonselective α_1-α_2-Antagonists

Phenoxybenzamine and phentolamine are examples of α-adrenergic antagonists with effects at both α_1- and α_2-adrenergic receptors. There are few clinical indications for the use of phentolamine in veterinary medicine and formulations of this drug are no longer consistently available in the United States.

Phenoxybenzamine binds covalently to α-adrenergic receptors and produces an irreversible block. Restoration of physiological responses to activation of α-adrenergic receptors following phenoxybenzamine administration requires the synthesis of new α-adrenergic receptors. The α-adrenergic antagonistic effects of phenoxybenzamine are more substantial with α_1- than α_2-adrenergic receptors. Phenoxybenzamine demonstrates a variety of other pharmacological effects including inhibiting the reuptake of NE at presynaptic nerve terminals and acting to various degrees as an antagonist at histamine, acetylcholine, and serotonin receptors. The predominant, and perhaps only, use of phenoxybenzamine in veterinary medicine is to manage the symptoms of catecholamine excess in the treatment of patients with a pheochromocytoma (Herrera et al., 2008; Agrawal et al., 2014).

Phenothiazine tranquilizers are also potent, nonselective α-adrenergic receptor antagonists. The CNS effects of these drugs underlie their use as tranquilizers; however, the decrease in peripheral vascular resistance secondary to antagonism of α-adrenergic receptors is clinically significant and should be considered when selecting these drugs for tranquilization or anesthetic premedication. Similarly, the tetracylic antidepressant trazodone, used for its sedative properties in dogs, can antagonize α_1-adrenergic receptors.

Selective α_1-Antagonists

Prazosin is a competitive antagonist with a marked selectivity for the α_1-adrenergic receptor. Historically, prazosin has been used for the treatment of hypertension or to reduce afterload in patients with heart failure, but there are now more efficacious therapies. Currently, prazosin is used to decrease resistance to urine flow in the proximal and prostatic urethra in dogs with functional urethral obstruction, prostatic hyperplasia, and idiopathic vesicourethral reflex dyssynergia, or to facilitate voiding in animals with upper motor neuron bladders (detrusor–external sphincter dyssynergia) due to spinal trauma or intervertebral disc disease (Fischer et al., 2003; Haagsman et al., 2013). The efficacy of this drug in the treatment of urethral obstruction in cats is debated because smooth muscle is confined to the proximal third of the cat urethra, whereas urethral obstructions in these animals generally occur in the distal urethra, which is composed of skeletal muscle (Hetrick and Davidow, 2013; Lulic et al., 2013). Terazosin, doxazosin, and alfuzosin are reversible α_1-adrenergic receptor antagonists with similar physiological effects to prazosin, but with different pharmacokinetic profiles. Specific information regarding the use of these drugs in veterinary patients is limited.

Tamsulosin and silodosin are α_1-adrenergic receptor antagonists that demonstrate a degree of α_1-adrenergic receptor subtype selectivity. Tamsulosin is a second-generation α_1-adrenergic receptor antagonist that was developed with the goal of decreasing the frequency of orthostatic hypotension. Tamsulosin has a higher affinity for α_{1A} and α_{1D} receptors than for the α_{1B} subtype. The α_{1A}-adrenergic receptor subtype is a primary adrenergic receptor subtype in the urethra and prostate of humans and dogs, whereas the α_{1B}-adrenergic receptor subtype is a principal subtype in vascular smooth muscle in these species (Kobayashi et al., 2009). Silodosin is a third-generation, highly selective α_{1A} receptor antagonist. Dogs with and without prostatic hyperplasia demonstrated similar effects between silodosin and tamsulosin on decreasing intraurethral pressure, but silodosin had less of an effect on blood pressure (Kobayashi et al., 2009). Silodosin suppresses phenylephrine-induced increases in intravesical ureteral pressure in dogs and may facilitate passage of distal ureteral stones (Kobayashi et al., 2010). Although orthostatic hypotension is not as clinically significant in veterinary patients, the potential for effects on peripheral vasculature when using these drugs should be considered.

Selective α_2-Antagonists

Yohimbine, tolazoline, and atipamezole are α_2-adrenergic receptor antagonists that are used to reverse the sedative effects of α_2-adrenergic agonists such as xylazine, medetomidine, dexmedetomidine, detomidine, and romifidine. Reversal of α_2-agonist-mediated sedation and off-label therapy of certain drug intoxications are currently the only therapeutic indications for these drugs in veterinary medicine.

Yohimbine was the prototype for selective α_2-adrenergic receptor antagonists and is an indolealkylamine alkaloid that is found in the bark of the tree *Pausinystalia yohimbe* and in *Rauwolfia* root. Yohimbine also demonstrates a degree of antagonistic activity at serotonin receptors. Yohimbine is used in veterinary patients to reverse sedation from α_2-adrenergic agonists, although less frequently in small animal patients since the introduction of atipamezole. The efficacy of yohimbine for reversal of xylazine sedation in cattle is variable.

Tolazoline belongs to the synthetic group of competitive α_2-adrenergic receptor antagonists known as the imidazoline derivatives. Tolazoline is a mixed α_1- and α_2-adrenergic receptor antagonist, and the approved product, Tolazine Injection, is licensed for reversing xylazine in horses. This product is used off-label to reverse other α_2-adrenergic agonists in equids, such as detomidine, and for the reversal of α_2-agonist sedation in other large animal species and wildlife, where the cost of atipamezole may be a concern (Powell, 1998). The use of tolazoline or other α_2-adrenergic receptor antagonists to reverse detomidine sedation is incomplete and transient.

Atipamezole is an α_2-adrenergic receptor antagonist with an imidazole structure and a binding affinity and α_2/α_1 selectivity ratio much higher than those of yohimbine or tolazoline. Atipamezole is not selective for subtypes of α_2-adrenergic receptors (Pertovaara et al., 2005). Atipamezole was originally approved to reverse the sedative and analgesic effects of medetomidine hydrochloride. Atipamezole rapidly reverses sedation induced by α_2-adrenoceptor agonists, at one-tenth the dose required for yohimbine. Atipamezole at clinically relevant doses causes few cardiovascular effects despite the potential increase in NE following blockade of presynaptic α_2-adrenergic receptors. Atipamezole reverses the bradycardic effects produced by α_2-adrenergic receptor agonists. Atipamezole has been used off-label in dogs to treat intoxication by imidazoline decongestants (e.g. oxymetazoline, tetrahydrozoline, xylometazoline) that are α_2-adrenoceptor agonists used topically for relief of nasal congestion or conjunctival redness. Atipamezole has also been used off-label to treat intoxication from the insecticide/acaricide, amitraz, which is an α_2-adrenergic receptor agonist (Bahri, 2008).

Clinical Use of α-Adrenergic Receptor

It is not uncommon for small animal patients to develop a urethral obstruction, a condition that is often relieved with a urethral catheter. Unfortunately, recurrence of urethral obstruction following removal of the urinary catheter is a frequent complication (Hetrick and Davidow, 2013). Activation of sympathetic nerves to the bladder facilitates relaxation of the bladder body smooth muscle through activation of β_2- and β_3-adrenergic receptors, and enhances contraction of the smooth muscle of the proximal urethra by activation of α_1-receptors.

Urethral spasm is thought to play a role in urethral obstruction recurrence and drugs that mediate urethral smooth muscle relaxation by α_1-adrenergic receptor blockade are a useful component of postobstruction therapy. The two most frequently used drugs in veterinary medicine are phenoxybenzamine and prazosin. Prazosin has a higher α_1-adrenergic receptor affinity, and administration of prazosin to dogs results in a greater reduction of urethral pressure than phenoxybenzamine (Fischer et al., 2003).

Important limitations to the use of α_1-adrenergic receptor antagonists for urethral relaxation are the cardiovascular effects. Vascular smooth muscle tone is reduced with α_1-adrenergic receptor blockade and significant decreases in systolic, diastolic, and mean arterial blood pressure can occur. Newer selective α_1-adrenergic receptor antagonists, such as silodosin, have less of an effect on blood pressure than nonselective antagonists like prazosin, but clinical data for use of α_{1A} selective antagonists in veterinary medicine at this point is limited.

Beta Adrenergic Receptor Antagonists

Beta-adrenergic receptor antagonists are used widely in human medicine because of their efficacy in the treatment of hypertension, ischemic heart disease, congestive heart failure, and certain cardiac arrhythmias. While some of these clinical entities are also indications for the use of β-adrenergic antagonists in veterinary patients, the routine use of β-blockers in veterinary medicine remains somewhat controversial based on the relative dearth of information determining the clinical efficacy of these drugs in veterinary patients.

β-adrenergic receptor antagonists are structurally similar to catecholamines and competitively reduce receptor occupancy by catecholamines and other β-adrenergic agonists. Most of the available drugs are pure antagonists; however, there are several that are partial β-adrenergic receptor agonists (e.g., pindolol and acebutolol). These drugs can cause partial activation of β receptors, although not to the level of full agonists such as EPI, and inhibit the activation of β-adrenergic receptors in the presence of high levels of EPI and NE. This effect of some β-adrenergic antagonists is often

Table 7.7 Pharmacodynamic characteristics of adrenergic receptor antagonists commonly used in veterinary medicine

Drugs	β-Receptor selectivity	α_1-Receptor block	Intrinsic sympathetic activity	Local anesthetic activity
Atenolol	β_1	No	No	No
Betaxolol	β_1	No	No	Slight
Carvedilol	None	Yes	No	No
Celiprolol	β_1	No	Yes	No
Esmolol	β_1	No	No	No
Metoprolol	β_1	No	No	Yes
Pindolol	None	No	Yes	Yes
Propranolol	None	No	No	Yes
Sotalol	None	No	No	No
Timolol	None	No	No	No

referred to as intrinsic sympathetic activity (ISA). Drugs with ISA maintain slight basal stimulation of the β_1- and β_2-adrenergic receptors, and this modest β-mediated activity may prevent profound bradycardia or negative inotropy in the resting heart, constriction of bronchioles, or up-regulation of β-adrenergic receptors that can result from long-term therapy with β-adrenergic receptor antagonists. Whether there are clinical advantages or indications for using a drug with ISA remains unclear.

Some β-adrenergic antagonists exhibit membrane-stabilizing activity, similar to local anesthetics, and other β-adrenoceptor blockers are classified as inverse agonists (e.g., carvedilol). Receptors exist in a conformational equilibrium between inactive and active states and this equilibrium shifts with the binding of a ligand. Inverse agonists favor the inactive conformation and will decrease the propensity of the receptor to assume a conformation that is required for stimulation of cAMP, thereby reducing the constitutive activity of the β-adrenergic receptor (Khilnani and Khilnani, 2011).

β-adrenergic receptor antagonists are frequently categorized by their affinity for β-adrenergic receptor subtypes, presence or absence of ISA, and their membrane stabilization effects (Table 7.7). Drugs differ in their relative affinities for β_1 and β_2 receptors. Some have higher affinity for β_1- than for β_2-adrenergic receptors, but none of the clinically available drugs are completely specific for a given β-adrenergic receptor, and the ultimate clinical effect will be dose dependent.

Cardiovascular Effects

The antagonism of β-adrenergic receptors can substantially affect cardiovascular regulation, although there are numerous factors that can influence these responses, including: species differences, human and animal differences, the presence of background cardiovascular disease, and the specific antagonist administered. General cardiovascular effects are reviewed here. Antagonism of cardiac β-adrenergic receptors slows atrioventricular conduction, and produces negative inotropic and chronotropic effects, which may mediate a reduction in cardiac output (Muir et al., 1996). Beta-blocking drugs when administered chronically may reduce blood pressure in hypertensive patients, although they generally do not reduce blood pressure in normotensive patients. It is important when reviewing the effects of individual drugs to distinguish between effects in normal subjects and subjects with cardiovascular disease. Increased peripheral vascular resistance is often an acute effect observed following administration of β-adrenergic receptor antagonists, an effect that is mediated in part by blockade of vascular β_2 receptors. However, with long-term use of β-adrenergic receptor antagonists, peripheral vascular resistance returns to initial values or decreases in patients with hypertension. The mechanism for this response is not well understood, but may involve an effect on the release of renin from the juxtaglomerular apparatus. Activation of the SNS innervation to the kidney increases renin release via activation of β_1-adrenergic receptors, and this effect is reduced with β-adrenergic receptor blockade. In addition, some β-blockers, such as labetalol and carvedilol, also demonstrate antagonistic properties at α_1-adrenergic receptors, which may contribute to a reduction in peripheral vascular resistance.

Pulmonary Effects

Nonselective β-adrenergic receptor antagonists will block β_2-adrenergic receptors in bronchial smooth muscle leading to bronchoconstriction and increased airway resistance. In normal subjects this effect is minimal but in patients with asthma or chronic obstructive pulmonary disease it can be life-threatening. The implications of this in veterinary medicine have not been explored. With regards to recurrent obstructive pulmonary disease, aerosol administration of propranolol or atenolol during an acute airway obstruction can reduce dynamic compliance, whereas during clinical remission the drugs may exert little effect. The clinical indications for the use of β-adrenergic receptor antagonists in equine patients are few, and the effects of β-adrenergic receptor antagonists in cats with feline lower airway disease have not been studied.

Effects on the Eye

Topical administration of β-adrenergic receptor antagonists reduce intraocular pressure by blocking NE released from sympathetic nerve endings from acting on β-adrenergic receptors located on the ciliary epithelium, thereby mediating a decrease in aqueous humor production.

Metabolic and Endocrine Effects

β-adrenergic receptor antagonists inhibit the activation of lipolysis and glycogenolysis produced by catecholamines and sympathomimetic drugs. Nonselective β-adrenergic receptor antagonists may delay recovery from hypoglycemia in patients with type 1 diabetes mellitus, an effect that appears to be less severe in type 2 diabetic patients. β_1-selective adrenergic receptor antagonists are less likely to inhibit the recovery of blood glucose levels from an acute bout of hypoglycemia.

Specific Drugs

Beta-adrenergic receptor antagonists are classified as nonselective β-adrenergic receptor antagonists (first generation), β_1-selective receptor antagonists (second generation), and nonselective or subtype selective β-adrenergic receptor antagonists with additional cardiovascular actions unrelated to β-adrenergic receptor blockade (third generation). The receptor specificity and other nonadrenergic-related effects of several β-adrenergic receptor antagonists are summarized in Table 7.7. Clinically relevant information for many of these drugs is lacking due to the fact that relatively few controlled trials have been completed in animals with naturally occurring disease. Drugs that have been used most frequently in veterinary patients include propranolol, atenolol, esmolol, metoprolol, and carvedilol.

Nonselective β-adrenergic Receptor Antagonists

Propranolol is the prototype nonselective β-adrenergic receptor antagonist and in veterinary medicine has historically been used in the treatment of tachyarrhythmias, hypertension, hypertrophic and obstructive cardiomyopathies, and to treat the cardiovascular consequences of thyrotoxicosis or pheochromocytoma. Propranolol is a competitive antagonist with no intrinsic sympathomimetic activity. It does have membrane-stabilizing activity, but the therapeutic significance of this is not well defined.

Propranolol is well absorbed from the gastrointestinal tract following oral administration, is lipophilic, and demonstrates a large volume of distribution. However, this drug undergoes extensive hepatic metabolism, therefore its bioavailability is low. With the development of more selective β-adrenergic receptor antagonists, the use of propranolol is rarely indicated.

Selective β_1-Adrenergic Receptor Antagonists

Metoprolol is a selective β_1-adrenergic receptor antagonist that exhibits no intrinsic sympathomimetic activity. Metoprolol has a large volume of distribution and undergoes extensive oxidative metabolism in the liver, with less than 10% excreted unchanged in the urine. The elimination half-life of metoprolol in small animals is much shorter than that reported in people. Atenolol is a selective β_1-adrenergic receptor antagonist with no intrinsic sympathomimetic activity, a half-life that is longer than that of metoprolol, a large volume of distribution, and is characterized by minimal hepatic metabolism. Esmolol is an ultra-short-acting β_1-adrenergic receptor subtype selective antagonist with a half-life of about 10 minutes. Steady-state concentrations are quickly achieved during continuous intravenous infusions and its effects are terminated rapidly when the infusion is discontinued.

Third-Generation Nonselective Antagonists

Carvedilol is a nonselective β-adrenergic receptor antagonist and demonstrates modest α_1-adrenergic receptor antagonist properties. Carvedilol attenuates oxygen free radical-initiated lipid peroxidation and has antiproliferative effects. Carvedilol demonstrates membrane-stabilizing activity but no ISA. Carvedilol is rapidly absorbed following oral administration and is extensively metabolized.

Clinical Uses of β-Adrenergic Receptor

Acquired Cardiac Disease in Dogs

Studies in heart failure patients have demonstrated that β-adrenergic receptor antagonism with β_1-selective antagonists or the third-generation antagonist, carvedilol, reduce mortality and/or hospitalization and increase quality of life (Packer et al., 1996; Bristow, 1997). Initially the use of β-adrenergic receptor antagonists in the treatment of heart failure was avoided due to their negative inotropic effects, and the potential for bradycardia, cardiac failure, hypotension, and bronchospasm. However, the availability of more β_1-adrenergic receptor selective drugs or those with additional nonadrenergic related cardiac effects have reduced the potential for some of these complications.

Chronic valvular heart disease, specifically chronic mitral valvular disease, is the most common cause of heart disease and congestive heart failure in dogs, followed by dilated cardiomyopathy (DCM). Neuroendocrine mechanisms that occur during the development of heart failure include stimulation of the SNS, activation of the renin–angiotensin system, and release of vasopressin. High circulating concentrations of NE are evident in dogs with clinical chronic degenerative atrioventricular valve disease (Ware et al., 1990) and in experimental models of mitral valve regurgitation (MR) (Tsutsui et al., 1994). Increased cardiac sympathetic nerve activity precedes the increase in circulating NE in subjects with primary MR. Although beneficial in the early stages to preserve inotropy, chronic sympathetic activation and elevated catecholamine levels can lead to cardiac hypertrophy and remodeling, myocyte necrosis, chronically elevated heart rate, elevated afterload, and cardiac arrhythmias. A protective adaptation of downregulation of β_1-adrenoceptors has been described. One of the proposed mechanisms of systolic dysfunction seen with primary MR from chronic mitral valve disease (CMVD) is related to the increased sympathetic activity which results in a reduction of the number of cardiomyocytes and the number of contractile elements within each cardiomyocyte.

It has been proposed that β-adrenergic receptor antagonists might be a beneficial therapy in dogs with CMVD. One study reported a significant improvement of the left ventricular function in dogs with experimentally induced MR treated with the β antagonist atenolol (Nemoto et al., 2002). The administration of atenolol resulted in a decrease in cardiac interstitial NE in the experimental dogs. However, in a retrospective study of dogs with acquired DCM or CMVD, metoprolol administration was associated with no significant differences in cardiac dimensions as measured with echocardiography (Rush et al., 2002).

The effects of orally administered carvedilol were studied prospectively in dogs with mitral valve disease (Marcondes-Santos et al., 2007). The primary end points involved quality of life and sympathetic activation with secondary end points involving echocardiographic variables. The study period was 3 months and there was concurrent administration of benazepril or benazepril and digoxin depending on the severity of disease. The dogs treated with carvedilol had improvement in quality of life scores and a modest reduction in systolic blood pressure. Carvedilol did not improve the sympathetic activation and echocardiographic variables over the 3 months of treatment. Similarly, in a prospective study of dogs with DCM, there was no difference in echocardiographic and neurohormonal variables in the group treated with carvedilol. Additionally, there was no difference in owner-perceived quality of life (Oyama et al., 2007).

Administration of β-adrenergic antagonists is reported to initially worsen hemodynamics and contractile function in dogs with experimental MR. Acute decompensation has been reported in people and dogs with heart failure immediately after initiation of therapy (Kittleson and Hamlin, 1981; Fung et al., 2003). More recently, the phosphodiesterase III inhibitor, pimobendan, has shown clear benefits in dogs with CMVD or DCM and the use of β antagonists in these patients is now seldom indicated (Haggstrom et al., 2008; O'Grady et al., 2008).

Hypertrophic Cardiomyopathy and Ventricular Outflow Obstruction

Hypertrophic cardiomyopathy (HCM) is the most common cardiac disease in cats and a significant percentage of cats with HCM will develop dynamic obstruction of the left ventricular outflow tract (LVOT). HCM and hypertrophic obstruction cardiomyopathy (HOCM) are characterized by concentric ventricular hypertrophy, dysfunction during diastole, and elevation of left ventricular end–diastolic pressure and left atrial pressure. Obstruction of the LVOT can be due to a single cause or a combination of causes that include asymmetric ventricular septal hypertrophy and anterior (cranial) motion of the mitral valve during systole (SAM).

The most commonly prescribed medications used to reduce dynamic LVOT obstruction in cats with HCM are β-adrenergic receptor antagonists, particularly atenolol. Echocardiographic evaluation of cats with HCM or HCOM given a single dose of atenolol revealed decreases in heart rate, peak velocity in the LVOT, systolic fractional shortening, and left atrial size. Atenolol consistently reduced, and in some cases relieved, LVOT obstruction (Blass et al., 2014). There are conflicting reports on the prognostic importance of SAM in cats with HCM and whether treatment with atenolol should be instituted in preclinical HCM remains controversial. It would be predicted that the negative chronotropic and inotropic effects would reduce peak velocity of blood flow at the LVOT which would, theoretically, decrease the force on the mitral valve leaflets against the septum and delay the development of SAM. The negative inotropic effect would decrease systolic fractional shortening and the potential for dynamic obstruction from the hypertrophied ventricular septum.

Subaortic stenosis (SAS) in dogs is a congenital cardiac abnormality that causes varying degrees of LVOT obstruction. The disease is characterized by an abnormal ridge of fibrous tissue below the aortic valve which results in a decreased cross-sectional area of the LVOT. The severity of disease and prognosis varies depending on the degree of stenosis and the subsequent pressure gradient across the stenosis. Dogs with a pressure gradient of less than 50 mmHg are considered to have mild disease, those with a gradient of 50–80 mmHg are classified as having moderate disease, and severe stenosis would be that which creates a gradient greater than 80 mmHg. Dogs with mild or moderate SAS are considered to have a good prognosis and no treatment is recommended unless symptomatic. Dogs with severe SAS have been empirically treated with beta blockers, most commonly atenolol. However, in a recent retrospective review, beta blocker treatment did not influence survival in dogs with severe SAS (Eason et al., 2014).

Cardiac Arrhythmias

Beta-adrenergic receptor antagonists are categorized as Class II antiarrhythmics and are used for treating supraventricular and ventricular tachyarrhythmias. β-adrenergic receptor blockade increases the atrioventricular nodal refractory period and slows ventricular response rates in atrial flutter and fibrillation, and reduces ventricular ectopic beats, particularly those precipitated by catecholamines. The β-adrenergic receptor antagonist sotalol has antiarrhythmic effects involving ion channel blockade and prolongs the action potential and extends the refractory period (class III antiarrhythmic).

Ocular Disease

Timolol maleate is a nonselective β-adrenergic receptor antagonist that lowers intraocular pressure by decreasing aqueous humor production. In normal dogs and cats, topical application of timolol results in reduction of intraocular pressure in the treated eye and the contralateral eye, as well as miosis in the treated eye in cats and in the treated and contralateral eye in dogs (Wilkie and Latimer, 1991a, 1991b). Timolol maleate is available in a 0.25% and 0.5% solution and dosing is every 12 hours. In general, the 0.25% solution is recommended for small dogs and in cats due to the possibility of systemic absorption of the drug and systemic effects. Potential systemic side effects of topical timolol include bradycardia, cardiac arrhythmias, heart block (β_1-adrenergic receptor blockade), and pulmonary effects such as exacerbation of asthma and bronchospasm (β_2-adrenergic receptor blockade). Topical timolol should be avoided in patients with cardiac or pulmonary disease. Betaxolol is a β_1-selective receptor antagonist, which has been shown to significantly delay the onset of glaucoma in dogs with primary closed angle glaucoma (Miller et al., 2000).

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8

Cholinergic Pharmacology: Autonomic Drugs

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The authors thank HR Adams for the original chapter upon which this is based.

Acetylcholine (ACh) is the primary neurotransmitter at autonomic ganglia, parasympathetic neuroeffector junctions, some sympathetic neuroeffector junctions, somatic neuromuscular junctions, the adrenal medulla (Figure 8.1 provides examples of each of these innervations), and certain regions of the central nervous system (CNS). In this chapter, drugs that influence postganglionic parasympathetic neuroeffector junctions and autonomic ganglia are examined.

Parasympathomimetic Agents

Cholinergic is used to describe nerve fibers that synthesize and release ACh without distinction as to anatomic site of action (Figure 8.2). Parasympathomimetic is used specifically to describe an ACh-like effect on effector cells innervated by postganglionic neurons of the parasympathetic nervous system (PSNS). The spectrum of responses to parasympathomimetic drugs is not entirely restricted to PSNS effects, and may include cholinergic actions throughout the body (Barnes and Hansel, 2004; Brown and Taylor, 2006; Westfall and Westfall, 2006).

Based on mechanism of action, drugs that produce parasympathomimetic effects can be divided into two major groups (Figure 8.3): direct-acting agents, which like ACh activate cholinergic receptors located on effector cells; and cholinesterase inhibitors, which allow endogenous ACh to accumulate and thereby intensify and prolong its action (Brown and Taylor, 2006). Similar compounds are also used as antiparasitics and insecticides, and anesthetics, areas fully described in later chapters of this text.

Cholinergic Receptors

Acetylcholine is the principal endogenous agonist at two primary types of cholinergic receptors, nicotinic and muscarinic. Nicotinic neural (NN) receptors associated with the autonomic nervous system (ANS) are present on postganglionic neurons in autonomic ganglia and mediate neurotransmission from preganglionic to postganglionic neurons in both the sympathetic nervous system (SNS) and the PSNS. NN receptors are also present on adrenal medullary chromaffin cells and mediate neurotransmission from preganglionic SNS neurons to adrenal medullary chromaffin cells. Nicotinic muscle (NM) receptors are involved in mediating signal transmission at the neuromuscular junction and are an essential component of the somatic nervous system. Nicotinic receptors are ligand-gated ion channels and contain five homologous subunits organized around a central pore (Stokes et al., 2015). Activation of these receptors initiates: a rapid increase in cellular permeability to selective cations (Na^+ and Ca^{2+}); cell membrane depolarization; and excitation of postganglionic ANS neurons, adrenal medullary chromaffin cells, or skeletal muscle fibers (Stokes et al., 2015).

Muscarinic receptors are located predominately at postsynaptic sites, such as the heart, gastrointestinal tract, glands, and urinary bladder, which are innervated by postganglionic parasympathetic nerves. Five subtypes of muscarinic receptors have been identified and many of the physiological functions associated with PSNS activation are mediated by muscarinic2 (M2) and muscarinic4 (M4) receptors. Muscarinic receptors are G protein-coupled receptors (GPCRs), and activation of these receptors may elicit an excitatory or inhibitory response (Calebiro et al., 2010; Jalink and Moolenaar, 2010; Ambrosio et al., 2011; Vischer et al., 2011; Latek et al., 2012; Duc et al., 2015).

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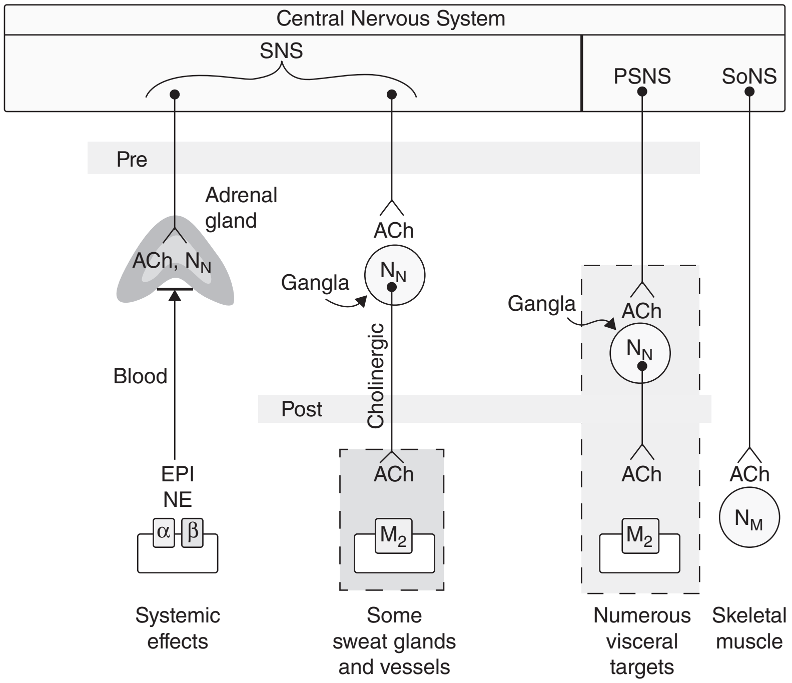


Figure 8.1 Schematic representation of the preganglionic and postganglionic anatomical relationships of nerves contained in the sympathetic nervous system (SNS) and the parasympathetic nervous system (PSNS). The anatomical substrate of somatic motor nerves (SoNS) is also shown. Only the primary neurotransmitters are shown. Acetylcholine (ACh) is the neurotransmitter released at sympathetic and parasympathetic ganglia and at most parasympathetic neuroeffector junctions. "Visceral targets" refers to cardiac muscle, glands, and bladder smooth muscle. Note that some sympathetic postganglionic fibers release ACh. The adrenal medulla, a modified sympathetic ganglion, is innervated by sympathetic preganglionic fibers and releases epinephrine and NE into the blood. ACh, acetylcholine; EPI, epinephrine; M, muscarinic receptors; NN, nicotinic receptors; NE, norepinephrine.

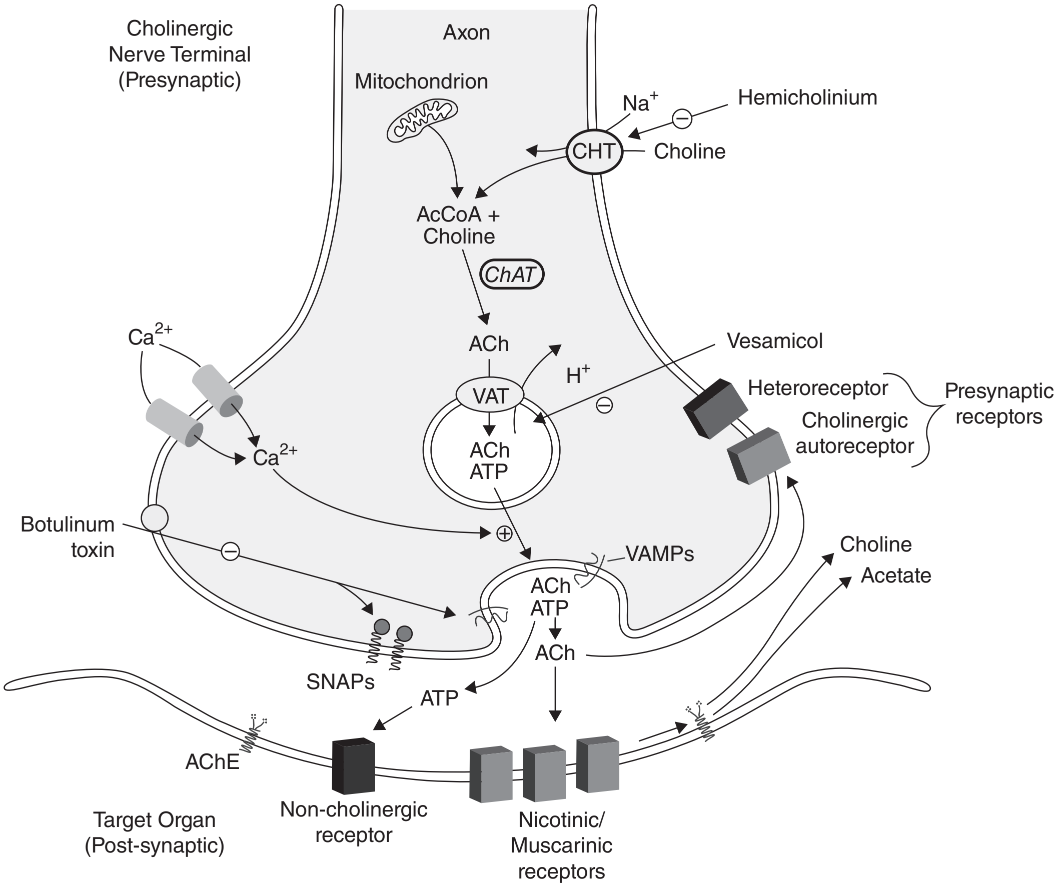
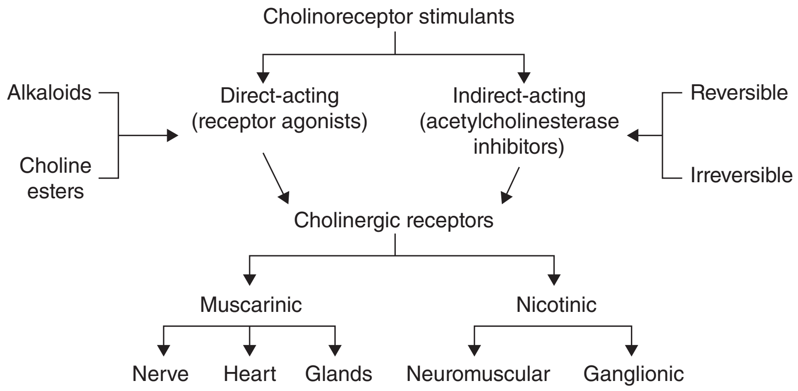


Figure 8.2 Schematic diagram depicting physiological processes at the site of a cholinergic nerve terminal innervating a target tissue. Processes have been described previously in Chapter 6. Cholinergic neurons synthesize and release ACh and this endogenous neurotransmitter binds to and activates nicotinic and muscarinic receptors. ACh, acetylcholine; AcCoA, acetyl-CoA; ChAT, choline acetyltransferase; CHT, choline transporter; SNAP, synaptosomal nerve-associated protein; VAT, vesicle-associated transporter; VAMP, vesicle-associated membrane protein.

Figure 8.3 Schematic summarizing primary cholinergic receptor stimulants, muscarinic and nicotinic receptors, and target tissues.



Direct-acting Parasympathomimetic Agonists

General Characteristics

Direct-acting parasympathomimetic agonists consist of choline esters, including ACh and numerous synthetic esters, and cholinomimetic alkaloids. Methacholine, carbachol, and bethanecol are primary choline derivatives, whereas muscarine, pilocarpine, and arecoline are primary cholinomimetic alkaloids. Pharmacological effects of ACh and related choline esters and alkaloids are mediated by activation of cholinergic receptors located on cells innervated by cholinergic nerves and, in some cases, on cells that lack cholinergic innervation. Direct-acting agonists act directly on receptors and do not depend upon endogenous ACh for their effects. In general, the physiological responses of selected organs and effector tissues elicited by activation of efferent parasympathetic nerves, as well as direct-acting parasympathomimetic agonists, are similar (Table 8.1). However, the pharmacological characteristics of direct-acting parasympathomimetic agonists demonstrate nonuniform susceptibility to metabolism by cholinesterases, differential relative affinity for muscarinic and nicotinic receptors, and specificity in target organ effects (Table 8.2).

Structure–Activity Relationships

Direct-acting cholinergic agonists contain structural groupings that allow interaction of the agent with cholinergic receptors and result in similar membrane and cellular responses to those caused by ACh. Chemical structures of several choline esters and cholinomimetic alkaloids are shown in Figures 8.4 and 8.5.

Choline esters contain a quaternary nitrogen atom to which three methyl groups are attached. Except for some naturally occurring cholinomimetic alkaloids, a quaternary nitrogen moiety is usually required for a direct potent action on cholinergic receptors. The quaternary nitrogen group carries a positive charge and this cationic group electrostatically binds with a negatively charged (anionic) site of the cholinergic receptor.

Receptive macromolecules (i.e., cholinergic receptors and cholinesterases) that recognize and bind ACh have, in addition to the anionic site, a region that combines

Table 8.1 Effects of direct-acting cholinergic receptor stimulants

Organ	Tissue	Response
Eye	Sphincter muscle, iris	Pupillary constriction
	Cillary muscle	Contraction
Glands	Salivary, lacrimal	↑↑ Secretion
Lung	Bronchial muscle	Contraction
	Bronchial glands	Stimulation
Heart	Sinoatrial node	↓ Heart rate
	Atria	↓ Contractility/conduction
	Atrioventricular node	↓ Conduction
	Ventricles	↓ Contractility (slight)
Blood vessels	Selected arteries	Dilation
Gastrointestinal tract	Motility	↑ GI Muscle Contraction
	Sphincters	↓ Tone
	Secretion	Stimulation
Urinary bladder	Detrusor muscle	Contraction
	Sphincters	Relaxation

Table 8.2 Scope of cholinergic receptor activating properties of some choline esters

	Agonistic properties						
	Susceptibility to cholinesterase		Muscarinic receptors				
	True	Pseudo	CV	GI	UB	E	Nicotinic receptors
Acetylcholine	+ + +	+ + +	+ + +	+ + +	+ +	+	+ + +
Methacholine	+	–	+ + +	+ +	+ +	+	±
Carbachol	–	–	+	+ + +	+ + +	+ +	+ + +
Bethanechol	–	–	±	+ + +	+ + +	+ +	–

CV, cardiovascular; GI, gastrointestinal; UB, urinary bladder; E, eye.

with the ester component of ACh (Hucho et al., 1991). In cholinesterase, this region is called the esteratic site and its combination with the carboxyl group results in hydrolysis of the ester. Hydrolysis of ACh does not occur upon its interaction with a receptor, however, and the ester-attracting region of the receptor is called the esterophilic site (Inestrosa and Perelman, 1990; Taylor, 1991, 2006a; Massoulie et al., 1993). ACh is structurally arranged so that it combines with the esterophilic and anionic sites of both nicotinic and muscarinic receptors and acetylcholinesterases (Hucho et al., 1991).

ACh is the prototypical cholinergic agent and activates both nicotinic and muscarinic receptors. Acetyl-β-methylcholine (methacholine) is identical in structure to ACh except for the substitution of a methyl group on the β-carbon atom of the choline group. This structural change yields a compound that is primarily a muscarinic receptor agonist lacking significant nicotinic effects when given in usual dosages. Further, it is more active on the cardiovascular system than on the GI tract. Duration of action of methacholine is considerably longer than that of ACh.

Carbachol and bethanechol each have a carbamyl group substituted for the acetic moiety of ACh, and bethanechol also has a β-methyl group. Both of these agents are almost completely resistant to inactivation by the cholinesterases. Their duration of action is therefore considerably longer than that of ACh. Carbachol is active at both muscarinic and nicotinic receptor sites, whereas bethanechol is primarily a muscarinic agonist. Unlike methacholine, both these drugs are somewhat more active on smooth muscles of the GI tract and urinary bladder than on cardiovascular function. Pharmacological characteristics of these choline esters are presented in Table 8.2.

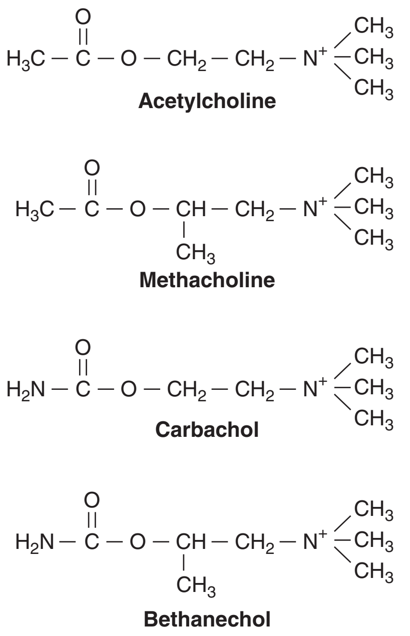


Figure 8.4 Molecular structures of primary choline esters.

Acetylcholine: Prototypical Cholinergic Agonist

Pharmacological Mechanisms and Effects

ACh is the prototypical cholinergic agonist and therefore provides a foundation for understanding the pharmacological effects of other cholinomimetic drugs. The

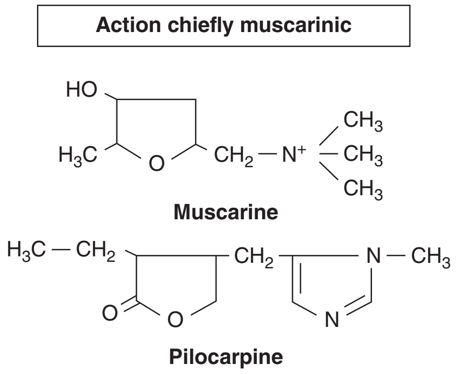


Figure 8.5 Molecular structures of several cholinomimetic alkaloids.

biosynthesis, neuronal release, cellular activities, and inactivation of endogenous ACh were discussed in Chapter 6. Although an essential ANS neurotransmitter, ACh is not used therapeutically for at least two reasons. First, muscarinic and nicotinic receptors are located at numerous tissue sites and therefore no selective therapeutic response to ACh can be achieved. Second, its duration of action is quite brief because it is rapidly inactivated by the cholinesterases. Several derivatives of ACh are more resistant to hydrolysis by cholinesterase and have a somewhat greater selectivity in their sites of action (Table 8.2).

Since ACh is a mixed nicotinic–muscarinic agonist, different physiological response profiles can be produced by administration of this agent, depending upon the relative dominance of muscarinic (parasympathomimetic) or nicotinic actions. These effects can be differentiated by use of small and large doses of ACh and by using selective cholinergic blocking drugs. In general, parasympathomimetic effects dominate with small doses, whereas with large doses nicotinic effects can be elicited. Use of cholinergic blocking drugs and small and large doses of ACh to differentiate muscarinic and nicotinic effects of ACh is shown in Figure 8.4. This figure is discussed in more detail in the following section regarding cardiovascular effects mediated by ACh administration.

Target Organ Effects of ACh

Cardiovascular: Intravenous (IV) administration of small amounts of ACh (5– 10 μg/kg) induces a brief but rapid fall in systolic and diastolic blood pressures, due to a decrease in peripheral resistance resulting from dilation of blood vessels. Most blood vessels receive little or no parasympathetic innervation, and muscarinic receptors located at these sites are noninnervated. Muscarinic receptors mediating dilation of blood vessels are located on the endothelium rather than on the smooth muscle, and the smooth muscle relaxation in response to ACh administration involves the production and release of nitric oxide (Furchgott and Zawadzki, 1980; Lowenstein et al., 1994).

Somewhat larger doses of ACh (10–30 μg/kg) produce pronounced muscarinic effects; therefore, marked reductions in peripheral resistance, heart rate, and blood pressure are observed. Atrial myocardial cells contain muscarinic receptors associated with vagal fibers, and activation of these receptors by ACh produces negative chronotropic and inotropic effects. Generally, the chronotropic effects predominate. In addition to its pronounced slowing effect on heart rate, ACh exerts important effects on impulse conduction.

With high doses (50–100 μg/kg) muscarinic effects of ACh on postganglionic effector cells are accentuated. Profound hypotensive and bradycardic responses are observed. Large doses of ACh produce, in addition to muscarinic (i.e., parasympathomimetic) effects, stimulation of the nicotinic receptors in autonomic ganglia (both parasympathetic and sympathetic) and the adrenal medulla. These effects are particularly evident when the muscarinic receptors of the parasympathetic neuroeffector junctions are blocked by atropine (nonselective muscarinic receptor antagonist). Under these circumstances large doses of ACh stimulate nicotinic receptors of both sympathetic and parasympathetic ganglia. However, because the muscarinic receptors of the parasympathetic neuroeffector junctions are blocked by atropine, the ACh released from postganglionic parasympathetic nerves does not bind to and activate the target organ muscarinic receptors. Under this condition, sympathomimetic responses will be evident, including increased arterial blood pressure, tachycardia, and other typical sympathetic-mediated effects. These effects can be blocked by use of appropriate adrenergic blocking drugs or by use of a ganglionic blocking agent (Figure 8.6).

Nonvascular smooth muscle: ACh stimulates smooth muscle of the urinary bladder and uterus to contract (Chapple et al., 2002). Bronchiolar smooth muscle is also contracted by ACh, resulting in decreased airway diameter (Barnes and Hansel, 2004; Fisher et al., 2004). The smooth muscle effects of ACh are due to muscarinic receptor activation.

Gastrointestinal system: Gastrointestinal motility and secretions are enhanced by ACh in a manner similar to that mediated by stimulation of the PSNS innervation to the gastrointestinal system. These effects may be difficult to detect with small doses because duration of action of ACh is brief owing to rapid destruction by cholinesterase. Larger doses markedly increase secretions and peristaltic movements of the GI tract.

Central nervous system: ACh does not readily cross the blood–brain barrier, therefore CNS effects are not observed when usual dosages are administered. However, intraarterial ACh injection into cerebral arteries or the direct application of ACh into the CNS produces central neural excitation. Both muscarinic and nicotinic receptors are present in the CNS (Krnjevic, 2004).

Adrenal medulla: The adrenal medulla is functionally analogous to autonomic ganglia, and nicotinic receptors located on adrenal medullary chromaffin cells are innervated by preganglionic sympathetic nerve fibers. These receptors are stimulated by ACh to cause release of epinephrine and norepinephrine from chromaffin cells into the circulation. This effect contributes to the overall nicotinic-mediated sympathomimetic effect evoked by large doses of ACh in the presence of muscarinic receptor blockade.

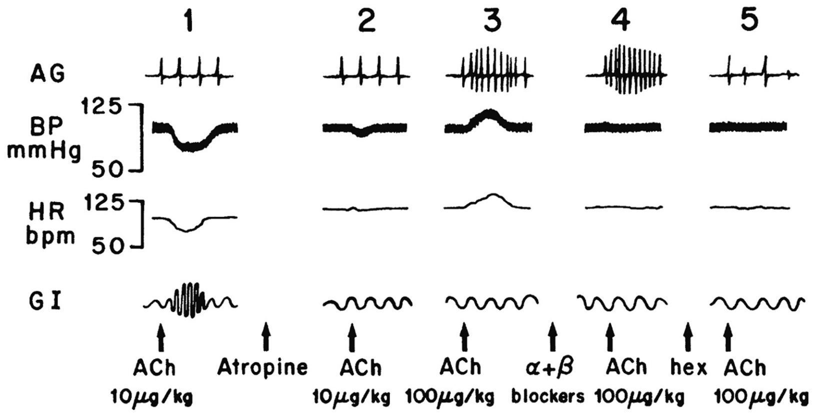


Figure 8.6 Muscarinic and nicotinic effects of acetylcholine (ACh) on blood pressure, heart rate, intestinal motility, and autonomic ganglionic action potentials in an anesthetized dog. Schematic reproductions: (1) A small dose of ACh (10 mg/kg) administered intravenously causes hypotension, bradycardia, and intestinal contractions caused by direct stimulation of muscarinic receptors of blood vessels, heart, and intestinal smooth muscle, respectively. These effects are brief because of rapid destruction of ACh by cholinesterase. (2) Atropine blocks the muscarinic receptors and thereby prevents the effects seen in (1). (3) Large doses of ACh (100 mg/kg) stimulate, in addition to muscarinic receptors, nicotinic receptors of parasympathetic and sympathetic ganglionic neurons, causing an increase in frequency and amplitude of ganglionic action potentials. Although all autonomic ganglia are activated, impulses arising from parasympathetic ganglia do not reach their effector cells because of blockade of parasympathetic postganglionic neuroeffector junctions by atropine. Sympathomimetic responses (pressor effect and tachycardia) result. (4) Impulses arising from sympathetic ganglia are prevented from reaching their effector cells by adrenergic blocking drugs; however, ganglionic nicotinic receptors are still activated by ACh. (5) Hexamethonium (hex) blocks nicotinic receptors of ganglia and thereby inhibits the nicotinic ganglionic stimulating effect of ACh and reduces ganglionic action potentials. AG, action potentials of autonomic ganglionic neuron; BP, systemic blood pressure; HR, heart rate; GI, intestinal peristaltic waves.

Choline Esters: Methacholine, Carbachol, and Bethanecol

Pharmacological Mechanisms and Effects

The pharmacological effects of methacholine, carbachol, and bethanecol are similar to the parasympathomimetic effects produced by ACh administration, and therefore are consistent with the physiological responses evoked by the activation of postganglionic parasympathetic nerves. However, the physiological response profiles produced by different choline esters are not identical, and vary in relative selectivity for one organ system or another (Table 8.2).

Methacholine is a synthetic choline ester that produces cardiovascular effects similar to those produced by ACh, but has a longer duration of action and its primary agonistic activity is at muscarinic receptors. Carbachol is active at both muscarinic and nicotinic receptors, and nicotinic neural receptors are particularly sensitive to carbachol. The pharmacological scope of activity of bethanecol is similar to that of methacholine and carbachol. Unlike carbachol, however, bethanecol is primarily a muscarinic agonist and has little stimulant effects on nicotinic receptors.

Target Organ Effects

Cardiovascular: Methacholine is more active on the cardiovascular system than on the GI or urinary tracts. The opposite selectivity is seen with carbachol and bethanechol. Intravenous administration of methacholine, like ACh, produces a depressor response and slowing of heart rate caused by activation of muscarinic receptors located on blood vessels and in the heart. Cardiac rhythm is altered by methacholine, and the atrioventricular node is particularly sensitive to this agent. Carbachol evokes blood pressure changes similar to, but less pronounced, than those produced by methacholine, whereas bethanechol administration produces considerably less effects on cardiovascular function.

Gastrointestinal system: Carbachol and bethanecol are relatively more active on the gastrointestinal and urinary tracts than on the cardiovascular system. Methacholine administration affects gastrointestinal function, but only in large doses. Carbachol is a potent GI stimulant and increases salivation and peristaltic movements of the gastrointestinal system.

Nonvascular smooth muscle: Carbachol causes contraction of bronchiolar smooth muscle, resulting in a decreased airway. The urinary bladder is contracted by carbachol and bethanechol. Effects of carbachol and bethanechol on smooth muscle are mediated by activation of muscarinic receptors.

Clinical Uses

There are few clinical indications for the use of these cholinergic agonists in veterinary medicine. Bethanechol has been used to promote bladder contraction in paraplegic dogs and cats with the goal to prevent over distension and detrusor muscle atony (Schubert, 2015). The use of bethanechol to promote detrusor muscle contraction for treatment of detrusor sphincter dysynergia, in conjunction with diazepam and prazosin to decrease urethral sphincter tone, has been reported (Jeyraja et al., 2010; Chandrasekar et al., 2013). The only clinical use for carbachol is as the ophthalmic solution Miostat®, which is administered at the end of cataract surgery to cause miosis.

Cholinomimetic Alkaloids: Pilocarpine, Muscarine, and Arecoline

Pharmacological Mechanisms and Effects

Pilocarpine, arecoline, and muscarine are plant alkaloids that are rather selective parasympathomimetic agents (i.e., their cholinomimetic activity is exerted primarily at muscarinic sites with minimal nicotinic effects). These cholinomimetic alkaloids evoke their parasympathomimetic effects by direct activation of muscarinic receptors.

Target Organ Effects

Pilocarpine is particularly effective in stimulating flow of secretions from exocrine glands, including; salivary, mucous, gastric, and digestive pancreatic secretions. As with ACh, it causes contraction of GI smooth muscle, thereby increasing smooth muscle tone and peristaltic activity. Of considerable importance, pilocarpine has a potent constrictor effect on the pupil.

Arecoline activates muscarinic receptors located at numerous targets including; glands, smooth muscles, and myocardium, and produces the usual parasympathomimetic effects. It is similar to pilocarpine in scope of activity.

Clinical Uses

Pilocarpine hydrochloride is available as a 1%, 2%, and 4% ophthalmic solution (see Chapter 49). Topical pilocarpine causes miosis and lowers the intraocular pressure. Although pilocarpine has been recommended historically to treat glaucoma, the topical irritation caused by this medication can be severe, making the medication poorly tolerated by most patients. Newer antiglaucoma medications are generally less irritating, and thus are recommended more commonly. Pilocarpine can be used to treat cases of neurogenic keratoconjunctivitis sicca in dogs. In order for pilocarpine to be effective there must be some normal functioning lacrimal tissue, and it is unlikely to increase tear production in cases of absolute keratoconujunctivitis sicca (Giuliano, 2013). Oral administration of pilocarpine should be performed with caution in small patients due to the increased risk of toxic systemic effects and death. Alternatively, a dilute pilocarpine solution (0.125% or 0.25%) can be applied directly to the eye to stimulate tear production. Pilocarpine can be used for pharmacological testing and neuroanatomic localization of mydriasis caused by dysfunction of the parasympathic nervous system. Ocular application of dilute pilocarpine solution (0.05%) in dogs with dysautonomia will cause rapid constriction of the pupil in less than 45 minutes compared to unaffected animals (O'Brien and Johnson, 2002). The rapid miosis is secondary to the degeneration of the postganglionic neurons, which leads to hypersensitivity of the denervated muscle to cholinergic drugs. In dogs with mydriasis due to a lack of parasympathic innervation of the iris sphincter muscle (internal ophthalmoplegia), the sphincter muscle will be hypersensitive to topical application of a dilute (0.1%) pilocarpine solution.

Cholinesterase Inhibitors

Pharmacological Mechanisms and Effects

Cholinesterase inhibitors (anticholinesterase agents) inactivate or inhibit acetylcholinesterase (AChE) and pseudocholinesterase, increasing the level of synaptic ACh, and intensifying the activity of endogenous ACh. Because cholinesterase inhibitors enhance the actions of endogenous ACh at all cholinergic receptors, their scope of activity is not limited to parasympathomimetic effects but can include cholinomimetic actions throughout the body (Taylor, 2006b). Effects of cholinesterase inhibitors can be reliably predicted by considering the anatomic location of cholinergic nerves and the respective physiological processes they modulate at target cells and tissues. Parasympathomimetic (muscarinic) effects of these agents are equivalent to the effects associated with activation of postganglionic parasympathomimetic nerves. Cholinesterase inhibitors also cause intensification of ACh activity at nicotinic sites.

Physostigmine, neostigmine, and edrophonium are examples of anticholinesterase drugs that produce a reversible inhibition of cholinesterase, whereas organophosphate compounds produce an irreversible inhibition. Although there is considerable distinction between these two groups of anticholinesterases, their pharmacological effects are similar because of a common mechanism of action. The pharmacological effects of cholinesterase inhibitors can be explained almost entirely by their characteristic inhibitory action on AChE. This results in decreased hydrolysis of neuronally released ACh and intensification of its action at cholinergic receptors. Neostigmine and some other quaternary nitrogen anticholinesterase agents exert some direct effects (either agonistic or antagonistic) on cholinergic receptors in addition to inhibition of cholinesterase.

Structure–Activity Relationships

The enzymatic interactions of AChE, ACh, and cholinesterase inhibitors can be summarized as follows (Inestrosa and Perelman, 1990; Taylor, 1991, 2006a; Massoulie et al., 1993). AChE contains two active sites that recognize specific parts of the ACh molecule: (i) an anionic (negatively charged) region where electrostatic binding occurs with the cationic nitrogen of the choline moiety; and (ii) an esteratic site where the carboxyl portion of the acetyl ester binds to it by covalent bonding. After ACh–AChE interaction occurs, the choline portion is split off, leaving the acetylated esteratic site. Acetic acid is rapidly formed as water reacts with the acetyl group, and the enzyme is thereby reactivated (Wilson, 1954).

Neostigmine, physostigmine, and other carbamate derivatives interact with the anionic and esteratic sites of the enzyme, thereby preventing ACh from affixing to the enzyme. Neostigmine and physostigmine are believed to be hydrolyzed in a manner similar to but much slower than that of ACh (Wilson et al., 1960). Although the rate of combination of inhibitor with AChE is only a few times slower than the analogous combination of ACh with the enzyme, the rate of hydrolysis is much faster for ACh. Therefore, neostigmine and related drugs are reversible cholinesterase inhibitors as a result of their actions as competitive substrates that are hydrolyzed at a much slower rate than the endogenous substrate ACh (Taylor, 1991, 2006a; Massoulie et al., 1993).

Edrophonium is a simple alcohol bearing one quaternary ammonium group. Edrophonium binds to the anionic site of cholinesterase by electrostatic attachment and at the esteratic site by hydrogen bonding. The action of edrophonium is brief because a covalent bond is not formed (Keegan, 2015).

Organophosphate compounds interact with AChE at the esteratic site and form an extremely stable enzyme–inhibitor complex that does not undergo significant spontaneous disassociation. The esteratic site is persistently phosphorylated, and recovery of cholinesterase activity is dependent upon de novo synthesis of new enzyme.

Reversible Inhibitors: Physostigmine, Neostigmine, Edrophonium, Pyridostigmine

Pharmacological Mechanisms and Target Organ Effects

As stated previously, these drugs produce their effects by combining with cholinesterase and thereby preventing the enzyme from hydrolyzing ACh. In general, the physiological responses produced by these drugs are similar to direct-acting parasympathomimetic agents, and therefore are consistent with the physiological responses evoked by stimulation of postganglionic parasympathetic nerves.

Digestive tract: Physostigmine and neostigmine cause contraction of smooth muscle, thereby increasing motility and peristaltic movements of the gut. Frequency and strength of peristaltic waves are increased, and movement of intestinal contents is accelerated.

Eyes: Physostigmine causes pupillary constriction when applied locally to the eye or when injected for systemic effect.

Skeletal muscle: Besides its major action of inactivating AChE at the somatic myoneural junction, neostigmine is believed to directly stimulate nicotinic receptors of skeletal muscle fibers. The skeletal muscle effects of neostigmine are relatively more pronounced at low doses than the smooth muscle effects of this agent. Twitching of skeletal muscles may be observed when a large dose of physostigmine or neostigmine is injected.

Other effects: A therapeutic dose of physostigmine or neostigmine does not produce pronounced effects on cardiovascular function. Effects of higher doses are complicated by concurrent ganglionic stimulation and muscarinic effects on the heart and blood vessels. Usually, hypotension and a bradycardia are produced. Smooth muscle of the bladder is cholinergically innervated and therefore is contracted by cholinesterase inhibitors. Bronchiolar smooth muscle is also contracted by these agents.

Clinical Uses

Physostigmine, pyridostigmine, neostigmine, and edrophonium can be used to reverse the effects of nondepolarizing neuromuscular blocking drugs in voluntary muscles. AChE inhibitors are used in the therapy of dogs but rarely cats, with myasthenia gravis (MG). Additionally, intravenous edrophonium is sometimes used as an initial screening test for dogs with suspected MG. MG results from either a rare congenital absence of nicotinic receptors at the neuromuscular junction or as an acquired autoimmune diseases resulting in destruction and deficiency of these AChRs. The edrophonium chloride response test is used to make a presumptive diagnosis. Edrophonium is used because of its ultrashort action. A positive response is defined as a temporary increase in muscle strength after administration of the drug (Khorzad et al., 2011). This is not a definitive test and both false-positive and false-negative results are possible. A definitive diagnosis of MG is made by documentation of autoantibodies against muscle AChRs by immunoprecipitation radioimmunoassay. Oral AChE inhibitors are used in the therapy for MG.

Unlike humans, spontaneous remission of acquired MG in dogs is likely (Shelton and Linstrom, 2001) and treatment is aimed at improving muscle strength while titrating the dose to minimize other cholinergic side effects. Pyridostigmine bromide and neostigmine bromide are the most commonly used AChE inhibitors for MG. Pyridostigmine is preferred because of its longer duration of action and lower potential for adverse gastrointestinal effects. In dogs with MG, pyridostigmine bromide is administered at 0.5–3.0 mg/kg orally two or three times daily (Khorzad et al., 2011). Adverse effects are related to the muscarinic effects of excess ACh and include increased gastrointestinal motility, diarrhea, salivation, and bradycardia.

Toxicology

Large doses of physostigmine first stimulate and then depress the CNS; small to moderate doses have little effect, whereas massive doses can produce convulsions. Neostigmine does not cross the blood–brain barrier to an appreciable extent. Toxic doses of these agents produce marked skeletal muscle weakness, nausea, vomiting, colic, and diarrhea. The pupil is markedly constricted and fixed. Dyspnea is characteristically seen from constriction of the bronchiolar musculature. Bradycardia and lowered blood pressure are also characteristic signs. Respiratory paralysis caused by depolarization block of the neuromuscular junction and compounded by excess bronchiolar secretions is the usual cause of death. Atropine is the most effective pharmacological antagonist for physostigmine or neostigmine toxicity.

Organophosphorus Compounds

Organophosphates irreversibly phosphorylate the esteratic site of both AChE and the nonspecific or pseudocholinesterase throughout the body. Endogenous ACh is not inactivated, and the resulting effects are due to the excessive preservation and accumulation of endogenous ACh (Gutmann and Besser, 1990; Taylor, 1991, 2006a). Organophosphate poisoning produces diffuse cholinomimetic effects: profuse salivation, vomiting, defecation, hypermotility of the GI tract, urination, bradycardia, hypotension, severe bronchoconstriction, and excess bronchial secretions. These signs reflect excess activation of muscarinic receptors of postganglionic parasympathetic neuroeffector junctions with typical parasympathomimetic actions.

In addition to the muscarinic effects, organophosphates produce skeletal muscle fasciculations, twitching, and, subsequently, muscle paralysis occurs. These effects are due to persistent excessive stimulation of the nicotinic receptors of skeletal neuromuscular junctions, resulting in the depolarizing type of striated muscle paralysis (Gutmann and Besser, 1990). Convulsions and frequently death are seen in organophosphate poisoning, caused by penetration of the agent into the CNS and subsequent intensification of the activity of ACh at CNS sites (Gutmann and Besser, 1990).

Atropine is a competitive antagonist to ACh and therefore administration of this muscarinic receptor antagonist can reduce the severity of the parasympathomimetic effects produced by organophosphates, and can also increase the quantity of organophosphate required to produce death. An atropine test can be completed if signs of organophosphate poisoning are present (Wismer, 2012).

Although phosphorylation of the esteratic site of cholinesterase by organophosphates yields a normally irreversible complex, certain compounds cause a disassociation of the enzyme link. Pralidoxime (pyridine-2-aldoxime-methiodide, 2-PAM) was synthesized based on structural requirements postulated by Wilson (1958) to be necessary for a selective antidote to the organophosphate–cholinesterase interaction. This compound causes an effective removal of the phosphate group from the enzyme, so the enzyme is reactivated. This oxime compound is an adjunct to atropine therapy in treating organophosphate poisoning.

Animals previously exposed to toxic doses of organophosphates experience considerable improvement after treatment with 2-PAM. Since 2-PAM significantly reverses the combination of organophosphate with cholinesterase, the reactivated enzyme can then perform its normal function. The phosphorylated enzyme complex tends to age with time and to become resistant to reactivation by oximes.

Muscarinic Receptor Antagonists

Muscarinic receptor antagonists block the effects of ACh and related cholinergic receptor agonists from binding to and activating muscarinic receptors; therefore they are termed antimuscarinic agents or muscarinic receptor antagonists. Both naturally occurring antimuscarinic compounds and synthetic antimuscarinic compounds are available for use. Atropine, the prototypical muscarinic blocking agent, is an alkaloid extracted from *Atropa belladonna* (deadly nightshade) and *Datura stramonium* (jimsonweed), whereas scopolamine (hyoscine) is found in *Hyoscyamus niger* (henbane). There are numerous synthetic drugs with antimuscarinic effects, including propantheline, glycopyrrolate, tropicamide, and butylscopolamine. The following sections focus primarily on atropine because it is the prototype muscarinic receptor-blocking agent.

Mechanism of Action

Muscarinic receptor antagonists interact with muscarinic receptors of effector cells and, by occupying these sites, prevent ACh from binding to the receptor. Physiological responses to parasympathetic nerve impulses are thereby attenuated. Blockade of muscarinic receptors of smooth muscle, cardiac muscle, and glands by atropine-like drugs involves a competitive antagonism and large doses of ACh or other cholinomimetic drugs (e.g., carbachol, cholinesterase inhibitors) can overcome inhibitory effects of atropine at these sites.

Antimuscarinic drugs, such as atropine and scopolamine, are considered nonspecific for different muscarinic receptor subtypes; however, the physiological effects of the drugs are somewhat dose and drug dependent. For example, salivary and cholinergic sweat glands are quite susceptible to small doses of atropine, whereas somewhat larger doses are required for blocking the effect of the vagus nerve on the heart. GI and urinary tract smooth muscles are less sensitive to atropine, and even larger dosages are required to inhibit gastric secretion. Except for effects on salivation and cholinergic sweating, it is difficult to achieve a selective action on targeted structures without concurrently inducing side effects on other, more susceptible sites. Net pharmacological effects of antimuscarinic drugs in a particular organ are influenced by the relative dominance of parasympathetic or sympathetic tone in that structure.

Pharmacological Effects

Cardiovascular system: An increase in heart rate and intranodal conduction velocity are the predominant effects of therapeutic doses of atropine. However, a dose-dependent decrease of heart rate and development of a second-degree atrioventricular block after treatment of bradycardia with atropine can occur. The effect of atropine on heart rate and the potential for subsequent tachycardia is dependent in part upon the degree of vagal tone. Because atropine blocks transmission of vagal impulses to the heart, animals with a preexisting high vagal tone would show a relatively greater tachycardia than those with low vagal tone.

Cardiac output may increase with atropine secondary to the increase in heart rate. Arterial blood pressure either remains unchanged or increases slightly as a result of the increased cardiac output. In animals exposed to exogenous ACh or other cholinomimetics (e.g., cholinesterase inhibitors), atropine can cause a relative increase in blood pressure, because muscarinic effects of the agonists will be blocked. Because atropine blocks the cardiac vagus, it markedly reduces or abolishes cardiac inhibitory effects of drugs acting through a vagal mechanism and will attenuate vagal-mediated reflex responses. Accordingly, the pressor effects of epinephrine and norepinephrine are accentuated in atropinized animals by blockade of the cardiac limb of vagal–baroreceptor reflexes.

Gastrointestinal system: Atropine causes relaxation of GI smooth muscle by inhibiting contractile effects of cholinergic nerve impulses. Thus, atropine and related drugs can be helpful in treatment of intestinal spasm and hypermotility. Inhibition of smooth muscle motility extends from stomach to colon, although the degree of blockade may not be uniform. Secretions of the GI tract are also blocked by atropine. Salivation is reduced quite markedly. Similarly, secretions of intestinal mucosa are inhibited; however, gastric secretions are reduced only with exceedingly high doses that also block virtually all other muscarinic sites.

Bronchioles: Cholinergic innervation to the bronchioles modulates secretion of mucus and contraction of bronchiolar smooth muscle via activation of muscarinic receptors. Atropine and other drugs of the belladonna group block effects of cholinergic impulses and thereby decrease secretions and increase luminal diameter of the bronchioles. The dilator action of atropine is valuable in counteracting constriction of bronchioles following overdosage of a parasympathomimetic drug.

Ocular effects: Atropine blocks the cholinergically innervated sphincter muscle of the iris and the ciliary muscle, resulting in mydriasis and cycloplegia after topical or systemic administration. Because atropine blocks cholinergic effects, adrenergic nerve impulses dominate and the pupil actively dilates. Atropine is contraindicated in the presence of increased intraocular pressure from glaucoma because the drainage system of the anterior chamber of the eye is impeded during mydriasis.

Urinary tract: Atropine relaxes smooth muscle of the urinary tract. The spasmolytic effect on the ureters may be of some benefit in treatment of renal colic. Atropine tends to cause urine retention because it inhibits smooth muscle tone.

Sweat glands: Atropine has a definite anhydrotic action in species such as humans, who have a cholinergic mechanism in control of sweat secretion, and a large dose may cause a hyperpyrexic response. Atropine does not directly affect sweating in species that have adrenergic mechanisms in control of sweating (e.g., equines) and has minimal effect in species that do not use cholinergic sweating as an important component of thermoregulation.

Central nervous system: Therapeutic doses of atropine produce minimal effects on the CNS (Fisher et al., 2004). Excessive doses may cause hallucinations and disorientation in humans and mania and excitement in domestic animals. Excessive motor activity followed by depression and coma is the usual sequence of events. Scopolamine has a slight sedative effect; when combined with morphine it produces analgesia and amnesia (referred to as "twilight sleep") in human patients. These effects of scopolamine usually are not detectable in domestic animals.

Clinical Uses

Muscarinic receptor antagonists can be used as antispasmodics or spasmolytics to control smooth muscle spasm. Antispasmodics can be used to decrease or abolish GI hypermotility and depress hypertonicity of the uterus, urinary bladder, ureter, bile duct, and bronchioles. Antimuscarinic drugs are not as effective as epinephrine or other adrenergic amines in dilating the bronchioles, but atropine is effective in antagonizing excessive cholinergic stimulation at these sites.

Systemically administered atropine is used predominantly to treat bradyarrhythmias that are associated with significant effects on cardiac output and/or systemic blood pressure. Given that the duration of effect on heart rate is approximately 30–60 minutes, this limits the use of atropine to initial or emergency treatment of bradyarrhythmias, or, more frequently, as a treatment for bradycardia that is induced by other drugs such as mu agonist opioids or acetylcholinesterase inhibitors. The routine use of anticholingergic drugs as anesthetic premedicants is no longer recommended, and treatment of bradycardia associated with drugs that markedly increase vascular resistance, such as dexmedetomidine may be contraindicated. Atropine may be administered as a diagnostic test to determine the responsiveness of a bradyarrhythmia to an anticholinergic drug.

Atropine is used to facilitate ophthalmoscopic examination of internal ocular structures and also for treatment of various ocular disorders. Atropine sulfate is available as a 1% ophthalmic solution and ointment and is a potent mydriatic and cycloplegic. Atropine is used commonly in the treatment of iridocyclitis in veterinary medicine because of its long duration of action. In dogs, the peak onset of mydriasis after application of 1% atropine solution is within 1 hour, and the mydriasis lasts for 96–120 hours (Rubin and Wolfes, 1962). Patients with dark irides may have a delayed onset and longer duration of atropine due to binding of atropine by the melanin in the irides, where it is slowly released from the pigment onto the muscarinic receptors (Salazar and Patil, 1976). A common side effect associated with the use of topical atropine is excessive salivation and vomiting, which is most likely due the bitter taste of the drug. After topical application, a portion of the solution will enter the nasolacrimal duct and may be ingested. This side effect is more likely to occur with use of the solution compared to the ointment formulation. Atropine can cause an increase in intraocular pressure and can decrease tear production, therefore it should be avoided in patients with glaucoma or keratoconjunctivitis sicca.

Diagnostic ophthalmoscopy is more frequently facilitated by topical tropicamide. Tropicamide is available as a 0.5% and 1% solution. The rapid onset and short duration of action make it an ideal drug for diagnostic ophthalmoscopy. Tropicamide has less cycloplegic effects than atropine, and is not commonly used to treat iridocyclitis.

Glycopyrrolate

The antimuscarinic effects of this synthetic muscarinic receptor antagonist are similar to atropine. It is often considered that glycopyrrolate may be associated with a lower risk of marked tachyarrhymias when compared to atropine. While clinical experience would suggest this is the case in veterinary patients, there is little research data to support this (Lemke, 2001). In dogs this compound effectively diminishes the volume and acidity of gastric secretions and reduces intestinal motility; it also reduces and controls excessive secretions of the respiratory tract. Similar control of respiratory secretions by glycopyrrolate has been reported in cats, and its duration of action exceeds that of atropine. Also, because of its more polar nitrogen moiety, glycopyrrolate penetrates the blood–brain barrier less effectively than atropine, with less propensity for unwanted CNS side effects.

N-butylscopolamine bromide

N-butylscopolamine bromide (NBB) is a quaternary ammonium that serves as a peripherally acting antimuscarinic, anticholinergic agent similar in action to atropine, and is approved in the United States for use in horses that demonstrate colic signs resulting from gas, spasms, or mild impactions. The efficacy of NBB for the treatment of spasmodic colic was based on a multicentered field study of naturally occurring simple colic in horses (Roelvink et al., 1991).

The elimination half-life of NBB in plasma is approximately 6 hours. The pharmacological effects produced by NBB are consistent with those of other anticholinergic drugs. In a study of the hemodynamic parameters, NBB decreased right atrial pressure, while cardiac output was maintained. The hemodynamic changes were similar to those reported following the administration of low doses of other anticholinergic agents such as atropine.

Recent work has focused on the potential utility of NBB administration for the treatment of marked airway obstruction that may occur with conditions of airway inflammation such as recurrent airway obstruction or heaves. Current evidence provides support for the use of NBB as an immediate bronchodilator (Couetil et al., 2012). Although tachycardia and reduced gastrointestinal motility are valid considerations, based on the short duration of activity and evidence to support improved ventilator function it is considered an appropriate therapeutic option when managing equine patients with severe airway obstruction. Additional evidence to support the use of NBB in horses suffering from airway obstruction is supported by a cross-over investigation in recurrent airway obstruction affected horses where atropine and NBB provided similar bronchodilatory effects, yet the systemic effects such as pupillary dilation and one horse developing colic signs were only observed following atropine administration (de Lagarde et al., 2014). From this study it was concluded that NBB bromide was associated with fewer systemic side effects and is therefore a preferred treatment for reversible airway obstruction in horses. See Chapter 48 for further discussion.

Methantheline, Propantheline, and Methylatropine

These drugs are quaternary amines used primarily as smooth muscle relaxants. Because of the charged quaternary group, these compounds do not cross the blood–brain barrier to an appreciable extent. Accordingly, they are considerably less effective than atropine as antagonists to organophosphates, since the CNS effects of the latter agents would not be blocked. In addition to muscarinic blocking effects, these drugs act as autonomic ganglionic blockers, which most likely contributes to their antispasmodic effect on GI smooth muscle. Propantheline has been used in dogs with myasthenia gravis that develop significant muscarinic side effects in response to the administration of pyridostigmine.

Autonomic Ganglionic Blocking Drugs

Following Langley's investigations in 1889, it was known that small doses of nicotine stimulate autonomic ganglion cells, and larger doses block the transmitter function of ACh at these same sites. Therefore, the cholinergic receptors located at autonomic ganglia, primarily on postganglionic neurons, have been classified as nicotinic. The nicotinic receptor represents the primary ganglionic transmission pathway present in all autonomic ganglia, although muscarinic receptors on postganglionic neurons can modulate ganglionic impulse transmission.

Nicotine is not used clinically in animals or humans as a ganglionic blocker; however, several drugs have been discovered that preferentially block autonomic ganglia by a competitive mechanism. These compounds interact with nicotinic neural receptors and block impulse transmission across the ganglionic synapse. Members of this group of ganglionic blocking agents include hexamethonium, pentamethonium, and chlorisondamine.

Because of the blockade of impulse transmission at the ganglia, ganglionic blocking drugs affect physiological responses at targets innervated by postganglionic fibers of the SNS and the PSNS. The overall effects of these agents on various functions are dependent upon the predominance of sympathetic or parasympathetic tone at a particular target, as indicated in Table 8.3 (Taylor, 2006b). Because the GI system functions predominantly under parasympathetic tone, ganglionic blockade often results in decreased motility and reduced secretions. Similarly, because heart rate is under dominant vagal tone, a relative tachycardia may result following ganglionic blockade. Because the smooth muscle tone of peripheral blood vessels is dominated by the SNS, vasodilation and hypotension occur after ganglionic block, and the output of catecholamines from the adrenal medulla is reduced.

Table 8.3 Usual predominance of sympathetic or parasympathetic tone in various tissues and consequent effects of autonomic ganglionic blockade

Structures	Predominant tone	Effects of ganglionic blockade
Eye		
Iris	Parasympathetic	Mydriasis
Ciliary muscle	Parasympathetic	Cycloplegia
Sweat glands	Sympathetic	Anhidrosis
Salivary glands	Parasympathetic	Dry mouth
Cardiovascular		
Arterioles	Sympathetic	Vasodilation: ↑ peripheral blood flow; hypotension
Veins	Sympathetic	Vasodilation: pooling of blood; ↓ venous return
Heart	Parasympathetic	Tachycardia
Gastrointestinal	Parasympathetic	↓ Tone and motility; constipation
Urinary bladder	Parasympathetic	Urinary retention

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Section 3

Anesthetics and Analgesics, and Drugs Acting on the Central Nervous System

9

Introduction to Drugs Acting on the Central Nervous System and Principles of Anesthesiology

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Drugs that act in the central nervous system (CNS) are of fundamental importance to health-care delivery. Some agents are administered to animals to directly improve their well-being. For example, without general anesthesia, modern surgery would not be possible. Some drugs alter behavior and improve animal–human interaction. They may induce sleep or arousal or prevent seizures. Drugs that act in the CNS are sometimes administered in an attempt to understand the cellular and molecular basis for CNS actions (i.e., physiology and pathophysiology) and/or identify the sites and mechanisms of action of other drugs. Finally, CNS actions of some drugs come as unwanted “side effects” when those drugs are used to treat conditions elsewhere in the body. For example, seizures may result from the injection of too much local anesthetic.

The first purpose of this chapter is to review principles of organization and function of the CNS. The intent is to lay a foundation from which later discussion on principles and applied aspects of CNS pharmacology can meaningfully follow. Behavior-altering drugs and anesthetics are routinely administered to animals by veterinarians and allied personnel. Appropriate use of these drugs is an important application of our knowledge of CNS pharmacology. Therefore, this chapter will conclude with a review of the principles of contemporary veterinary anesthesiology.

Introduction to CNS Drugs

Neuroanatomy and Neurophysiology

The CNS is largely the same anatomically across mammalian species. The brain and spinal cord have evolved to collect information about external and internal changes and to provide integration of this information in such a way as to promote the survival and reproduction of the animal. The information is gathered by sensory neurons that transduce a stimulus (e.g., light, sound, gas in the intestine) to an electrical signal (neuronal depolarization) that is transmitted to the central nervous system. The CNS then interprets this signal, computes a response, and initiates an output to effect an appropriate action (if needed). This response may need very little CNS integration or may need much greater interpretation. For example, an animal that steps on a red hot object stimulates a reflex arc to initiate an immediate withdrawal of the limb without any conscious perception that the object was hot. On the other hand a cat faced with a juicy steak sitting on a kitchen counter must first recognize the steak for what it is, and then compare that with its memory for what such an object might taste like and balance this with the memory of the punishment it received the last time it tried to steal such an object from that location. These latter computations involve complex integration in the CNS, and the neuronal involvement is largely controlled by chemical substances that cause, modify, or inhibit the depolarizations that lead to the final outcome.

Although the CNS of most mammals has the same basic organization, it is not surprising that evolutionary pressures have created differences in the relative size of certain components. This is easily demonstrated by examining the olfactory lobes of the brain in a human being and a dog, the latter having a much greater relative size than in people. There are also chemical differences in the CNS of different species that are not as readily explainable but clearly have an impact on our use of drugs to modify CNS activity. It is well known that opioids can cause excitation in horses and sedation in dogs and the distribution of mu and kappa receptors is very different between the two species (Hellyer et al., 2003).

In order to understand how drugs may modify activity within the CNS it is necessary to understand the normal processes that are used to alter interactions between cells within the CNS. To modulate incoming signals, mechanisms are necessary that allow acceptance or rejection of a particular impulse. In a few areas within the mammalian CNS there are neurons that are joined by electrical synapses. These synapses are characterized by tight

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connections between the two cells with ion channels that are aligned between the two cells. This arrangement does not allow the receiving cell to change the signal since depolarization of the first cell will result in depolarization of the second cell. However, this arrangement is useful where it is important that a number of cells fire simultaneously to produce a coordinated response and, since the signal can travel in either direction, it enhances the capability of this system to function in this way. The signal is also transmitted much more quickly between the cells (0.1 ms) so it can generate a very rapid response. Brainstem neurons involved in the coordination of breathing have this type of synapse and so do some neurons involved in the secretion of hormones from the hypothalamus. This electrical coordination allows them to produce a "pulse" of hormone by all firing simultaneously.

The vast majority of the neurons in the mammalian CNS, however, communicate by means of chemical signals. The depolarization of the first neuron travels down its axon by virtue of the opening of voltage gated sodium channels (Figure 9.1). At the synapse this wave of depolarization activates voltage-gated calcium channels that allow calcium to flow down the concentration gradient (approximately 10^{-3}M outside the cell to 10^{-7}M inside the cell) into the cytosol of the synapse. The presence of this increased concentration of calcium activates the discharge of vesicles containing a chemical messenger (neurotransmitter) into the synaptic cleft. This neurotransmitter diffuses across the small gap between the neurons and attaches to receptors on the postsynaptic membrane where it might produce one or more of four changes. It might cause a slight depolarization, but not enough to trigger an action potential; it might cause enough depolarization for the generation of an action potential (excitation); it might cause modifications to the internal milieu of the cell such that it could be more or less receptive to further signals (modulation); or it might cause an ionic shift resulting in hyperpolarization of the cell (inhibition). It is then necessary to have some mechanism to terminate that signal so that the postsynaptic neuron can return to its resting state to allow further signals to be received. This can occur by destruction of the neurotransmitter or its uptake into the presynaptic terminal or other surrounding cells.

Transduction

In order for an animal to experience its environment it must be able to convert multiple different forms of incoming energy into neuronal depolarization. This has led to the evolution of a large number of specialized receptors and chemicals that can transduce the incoming signal into something understandable by the animal. Photoreceptors in the eye use rhodopsin and a variety of opsins that are activated by light and amplified by connecting with a G protein-coupled receptor (GRPC). The mechanisms by which the specialized mechanoreceptors in the skin, the Meissner and Paccinian corpuscles, transduce stimuli are still not completely understood. The nerve terminals in these receptors have a variety of potassium and sodium channels that may be involved but the details have yet to be discerned. Nociceptors are known to use multiple receptors to transduce the noxious stimuli into electrical impulses. They respond to direct stimuli such as heat as well as chemical stimuli released from damaged tissue. The transient receptor potential vallinoid (TRPV) family of receptors (TRPV-1, TRPV-2, TRPV-3, TRPV-4) are involved in the transduction of thermal stimuli and respond to specific ranges of temperature (e.g., TRPV-3 responds to temperatures between 31 and 39°C vs. TRPV-1 to temperatures >42°C). The TRPV-1 receptor is also important in peripheral sensitization and in chronic pain states as it is upregulated in these conditions. Capsaicin is a TRPV-1 agonist and, because it continues to activate the channel, may lead to excessive calcium influx into the cell and cell death. In humans, a capsaicin patch has been developed that is applied to localized areas of neuropathic pain (e.g., postherpetic neuralgia) and can significantly reduce pain in some patients for several months after application(Wagner et al., 2013). Another TRPV-1 agonist, resiniferatoxin, has been used to treat osteosarcoma pain in dogs (Brown et al., 2005). There is a great deal of interest in TRPV-1 agonists and antagonists for the treatment of pain but this receptor is also involved in thermoregulation so systemic application of some of these drugs can lead to hyper- or hypothermia. As indicated in Figure 9.1, there are many other receptors on nociceptors and some of these will also tend to hyperpolarize the nociceptor making it less likely to fire. Examples of this include presence of opioid and α_2-adrenergic receptors on some of these neurons, which supports the action of these classes of drugs as peripherally acting analgesics. The peripheral nociceptors are also affected by mediators released as a result of tissue damage. These mediators may not directly cause depolarization but may sensitize the nociceptor to other stimuli leading to the phenomenon of peripheral hyperalgesia. These inflammatory mediators include prostaglandins, bradykinin, histamine, serotonin, cytokines (e.g., IL1β, TNFα), nerve growth factor, purines, endothelin, and protons. Many of these mediators have been pharmacological targets in the management of acute and chronic pain.

Presynaptic Processes

The cellular mechanisms that occur to allow these events to happen are complex and involve many steps. The neurotransmitters involved can be classed into three basic groups: the amino acids, the amines, and the peptides.

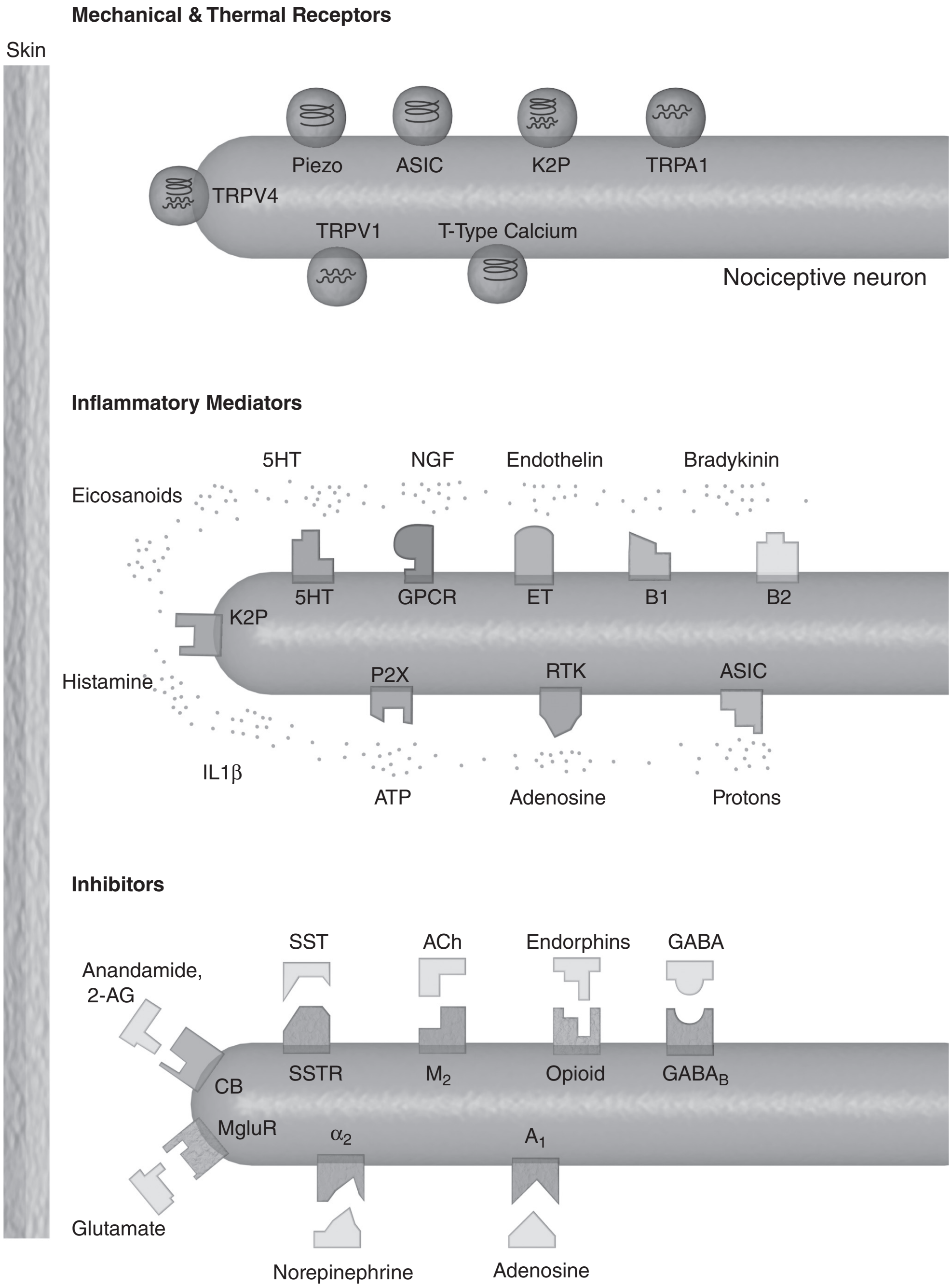

Figure 9.1 Transduction of nociceptive information by free neuronal endings in the dermis. Many receptors are depicted in this figure and it should be recognized that not all nociceptors would have all of these on their surface. (**A**) Receptors that provide direct transduction of thermal (wavy lines) and mechanical (spiral) stimuli. TRPA1, transient receptor potential A1, which responds to noxious cold; K2P, two-pore potassium channel; ASIC, acid-sensing ion channel, which responds to mechanical stimuli as well as protons; Piezo, responds to mechanical stimuli; TRPV4 and TRPV1, respond to hot thermal stimuli; T-Type calcium channel responds to mechanical stimuli. (**B**) Inflammatory mediators that may increase the likelihood of a nociceptor depolarizing. B1 and B2, bradykinin receptors; ET, endothelin receptor; GPCR, G protein-coupled receptor; 5HT, 5-hydroxytryptamine; P2X, purinergic receptor-2X; RTK, receptor tyrosine kinase; NGF, nerve growth factor; IL1β, interleukin-1β; ATP, adenosine triphosphate. (**C**) Inhibitory mediators that may decrease the likelihood of a nociceptor depolarizing. GABA, γ-amino butyric acid; Ach, acetylcholine; M2, muscarinic receptor-2; SST and SSTR, somatastatin and its receptor; CB, cannabinoid receptor; 2-AG, 2-arachidonylglycerol, a cannabinoid agonist; MGluR, metabotropic glutamate receptors; α_2, α-2 adrenergic receptor; A_1, adenosine receptor.

Amino acids can be absorbed from the extracellular fluid but usually involve active transport molecules to achieve this and some are immediately ready to use in this form. The amines and γ-amino-butyric acid (GABA) need to be synthesized from smaller building blocks that require the presence of enzymes. These enzymes are generally made in the cell body and diffuse slowly down the length of the axon to the axon terminal (Figure 9.2). This slow axonal transport occurs at 0.5–5 mm/day. Once an amino acid or amine is present in the nerve terminal it is packaged into synaptic vesicles (40–60 nm in diameter) using an active transporter system. For these small molecules the synaptic vesicles are seen as small clear-core vesicles on electron microscopy. The peptides, on the other hand, are manufactured in the cell body and packaged into larger vesicles at this site. They are actively transported to the axon terminal using microtubules and ATP-requiring proteins such as kinesin to achieve this (Figure 9.2). These large dense-core vesicles (appearance on electron microscopy) move at about 400 mm/day down the axon, so this occurs much faster than the diffusion of the enzymes needed for GABA and amine production. The synaptic vesicles tend to cluster around dense areas of the synaptic membrane (called *active zones*) that contain the necessary proteins to achieve transmitter release. The active zones have calcium channels that provide the stimulus for activation of the cascade required for docking and exocytosis of the synaptic vesicle. One

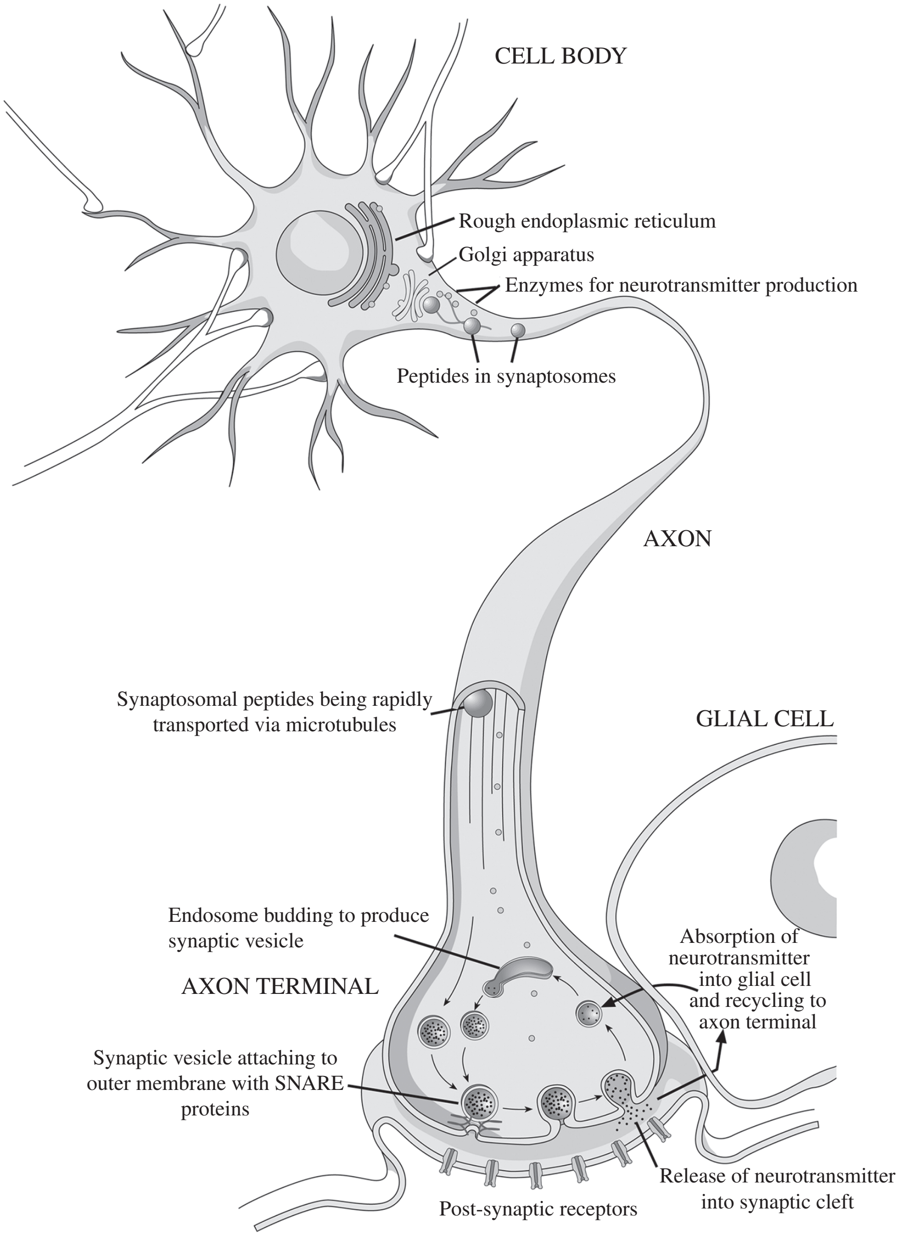
CELL BODY
Rough endoplasmic reticulum
Golgi apparatus
Enzymes for neurotransmitter production
Peptides in synaptosomes
AXON
Synaptosomal peptides being rapidly transported via microtubules
GLIAL CELL
Endosome budding to produce synaptic vesicle
AXON TERMINAL
Absorption of neurotransmitter into glial cell and recycling to axon terminal
Synaptic vesicle attaching to outer membrane with SNARE proteins
Release of neurotransmitter into synaptic cleft
Post-synaptic receptors

Figure 9.2 Neuron that is involved in chemical transmission. Enzymes and peptides are made in the cell body and transported to the axon terminal. The transport of enzymes is slow, whereas peptide synaptic vesicles are transported actively down the microtubules. Neurotransmitters are packaged into synaptic vesicles and these accumulate near the synaptic cleft. When the cell depolarizes the synaptic vesicles merge with the outer cell membrane and release neurotransmitter into the synaptic cleft. This chemical acts on the receptors on the postsynaptic membrane to alter postsynaptic cell function. Some neurotransmitters are destroyed in the cleft, some are reabsorbed into the axon terminal, and some may be absorbed by neighboring glial cells and destroyed or recycled into the axon terminal.

family of these proteins, referred to as the SNARE proteins (soluble *N*-ethylmaleimide attachment protein receptors) form complexes between the synaptic vesicle and the cell membrane (docking) and help promote the next step in the process (fusion of the membranes) so that the contents of the vesicle can be ejected into the synaptic cleft (exocytosis). These proteins are cleaved by botulinum and tetanus toxin, thus preventing neurotransmitter release (Breidenbach and Brunger, 2005). In the case of botulinum toxin, this effect remains peripheral at the neuromuscular junction whereas tetanus toxin is transported to the CNS where its effects occur mainly in inhibitory neurons, thus explaining the different manifestations of the two diseases (paralysis with botulinum and muscular rigidity with tetanus). The synaptic vesicles may then be reformed (endocytosis) with the aid of proteins called *clathrins* and recycled to be filled again with more neurotransmitter. The whole process from formation, exocytosis to endocytosis, and refilling of these synaptic vesicles can be carried out in about 1 minute, thus enabling frequent signaling from the terminal. Many synaptic terminals contain both small clear-core (amino acids and amines) and large dense-core vesicles (peptides). The peptide-containing vesicles are not usually released as readily as the clear-core vesicles and require a greater concentration of intracellular calcium for their release (usually the result of rapid repetitive depolarization of the neuron), thus providing the potential for a different signal to be associated with a more intense stimulus. Many presynaptic terminals have receptors on them that respond to the neurotransmitter being released (autoreceptors). This is usually presented as a feedback loop, which results in the inhibition of further release of the neurotransmitter.

Postsynaptic Processes

The receptors on the postsynaptic membrane can be divided into two types: the ionotropic or ligand-gated receptors and the metabotropic receptors. Ionotropic receptors allow the immediate passage of an ion across the cell membrane. For the most part, these are proteins that allow passage of sodium, calcium, potassium, or chloride. The metabotropic receptors activate a process inside the cell that may alter ionic conduction through another channel or they may alter the production of other substances within the cell that could change how the cell reacts to further stimulation. This process is very powerful because it can amplify the initial signal received. One receptor might activate a cellular enzyme that then catalyzes the production of many molecules, and each of these may further amplify the effect by activating further reactions. Normally, the signal from an ionotropic receptor is seen almost immediately, whereas the effect from a metabotropic receptor may take longer and may last longer after the stimulus.

In the CNS an individual neuron may receive input from hundreds of other neurons. Each synapse with its neurotransmitter can either excite (increase the resting membrane potential toward 0) or inhibit (decrease the resting membrane potential to a more negative value). The final effect on that individual neuron will be a result of the type of input it receives and the relative timing of that input. If there is simultaneous input from several excitatory neurons at the same time, it is likely that the neuron will depolarize and send a signal on to the next cell in the pathway. This is referred to as spatial summation. On the other hand, if a single input kept on firing it might achieve enough depolarization to trigger an action potential. This is referred to as temporal summation. If this neuron had received several signals from inhibitory neurons during this period the magnitude of the excitatory signal would have to be greater in order for the neuron to achieve a threshold for the action potential. Note, however, that inhibition under these conditions is not normally absolute and that if a strong enough stimulus is applied it will overcome the inhibition.

The attachment of a neurotransmitter to a receptor could be followed by a number of events. The receptor could have an effect and then the neurotransmitter and the receptor could be absorbed into the cell, thus terminating this effect (receptor internalization). The neurotransmitter could be destroyed by a chemical reaction catalyzed by an enzyme located nearby in the synaptic cleft. Some of the fragments of the molecule could then be absorbed again into the presynaptic terminal and reassembled into the original neurotransmitter while some fragments diffuse into the surrounding extracellular fluid. Lastly, the molecule could diffuse off the receptor as the concentration gradient reverses and it could be taken up into the presynaptic terminal or into surrounding glial cells for further transport back into the presynaptic terminal. These actions usually require the presence of transporter proteins for maximal efficiency.

The Neurotransmitters and their Receptors

There are a number of criteria for establishing a chemical as a neurotransmitter and a great deal of effort has been taken to ensure that these criteria are met by potential candidates. Just finding a chemical in the CNS does not mean that it is a neurotransmitter.

1) The substance must be present in the presynaptic terminal.
2) The substance must be released into the synaptic cleft when the presynaptic terminal depolarizes. In most instances this should be dependent on the intracellular release of calcium.

3) There should be receptors for the substance on the postsynaptic membrane that can be activated by exogenous placement of the substance on those receptors.

In the past, this kind of proof was difficult to obtain because an individual synapse is so small and being able to record from it is so technically challenging. Even with molecular biological techniques it is not simple but there have been a number of instances where a receptor has been discovered before the neurotransmitter that binds to it.

The Amino Acids

Glutamate: Glutamate is generally considered to be an excitatory neurotransmitter and is used in about half of the synapses in the brain. It is a nonessential amino acid that does not cross the blood–brain barrier so it has to be synthesized by the neuron (Table 9.1). Once it has been released into the synaptic cleft it is removed via a high-affinity uptake process on the presynaptic terminal and local glial cells involving excitatory amino acid transporters (EAATs). So far, five of these EAATs have been identified and their locations in the brain and relative activities vary (Bridges and Esslinger, 2005). The glutamate taken up in glial cells is converted to glutamine, which can then be absorbed by the presynaptic terminal and converted back to glutamate. Both EAATs and vesicular glutamate transporters (VGLUTs) have become targets for pharmacological manipulation. Glutamate acts on three families of ionotropic receptors and three classes of metabotropic receptors. The ionotropic receptors are named after the agonists that were first shown to activate them: *N*-methyl D-aspartate (NMDA receptor); α-amino-3-hydroxy-5-methylisoxazole-4-propionate (AMPA receptor); and kainate (kainate receptor). As with most of these receptors, they are composed of four or five subunits of membrane-spanning proteins and this gives rise to the possibility of many forms for each receptor (Table 9.1). These variations provide a basis for pharmacological targeting of individual receptors. The metabotropic receptors are divided into three classes, with class I (mGluR1 and 5) acting via $G_{q/11}$ proteins to increase phospholipase C (excitatory) and classes II (mGluR2–3) and III (mGluR4, mGluR6–8) acting via G_i/G_o proteins to inhibit adenylyl cyclase activity (inhibitory). So, although the major effects of glutamate are excitatory, there are parts of the brain where it can act as an inhibitory neurotransmitter. The NMDA receptors are important in the practice of anesthesia and analgesia because drugs that are antagonists at this receptor (cyclohexanones such as ketamine and tiletamine) are commonly used. At least six binding sites have been identified on the NMDA receptor for pharmacological activity. The site that binds glutamate (1) opens the channel to the entry of sodium and calcium into the cell. This site appears to need glycine to be bound to the receptor (2) for the glutamate to be fully effective. A third site within the channel binds phencyclidine and other cyclohexanones (3). There is also a voltage-gated magnesium binding site (4) within the channel; the ejection of magnesium from this site with depolarization opens the channel for further activity. There are also an inhibitory divalent site that binds zinc (5) near the mouth of the channel and a polyamine regulatory site (6) that potentiates the currents generated from the receptor when it is activated. The NMDA receptor is thought to affect long-term potentiation, memory, and plasticity of the nervous system. Long-term potentiation is likely involved with the modulation of nociceptive input. Activation of the receptor is also involved in the process of excitotoxicity leading to neuronal death.

Glycine: This is an inhibitory neurotransmitter, which is found in high concentrations in the medulla and spinal cord. Because glycine is found in all tissues in the body it has been difficult to isolate its activity in the CNS. The glycine receptor is a pentameric structure, and four α-subunits and one β-subunit have been identified. It is a ligand-gated chloride channel allowing the ingress of chloride ions, making the inside of the cell more negatively charged (hyperpolarization). The glycine and strychnine binding sites are located on the α_1 subunit. Once released into the synaptic cleft, glycine is taken back up into the presynaptic terminal or into surrounding glial cells by an active transporter for glycine (GLYT1-2), with GLYT-2 being the version mainly expressed on neurons. As indicated above, glycine interacts with glutamate at the NMDA receptor and GLYT is found in those neurons despite a lack of glycine receptors.

GABA: This is the major inhibitory neurotransmitter within the CNS and is present in as many as one-third of all synapses in the CNS. By comparison with its concentrations in the CNS it is found only in trace amounts elsewhere in the body. GABA is made from L-glutamic acid and this reaction is catalyzed by glutamic acid decarboxylase (GAD); this irreversible reaction needs a cofactor – pyridoxal phosphate (PLP a form of vitamin B_6). There are two forms of GAD (GAD_{65} and GAD_{67}) and GAD_{65} has a higher affinity for PLP, making its activity more easily regulated. GABA is broken down to succinic semialdehyde by GABA-transaminase (GABA-T), which also requires PLP and this can then enter the Krebs cycle via further breakdown to succinic acid. Since both GAD and GABA-T are dependent on PLP, a diet deficient in vitamin B_6 can lead to a decreased level of GABA in the brain with resulting seizures. GABA may be broken

Table 9.1 Small molecule neurotransmitters with their origins, transport proteins, method of catabolism, and receptors

Neurotransmitter	Synthesized from	Transport of raw material into presynaptic terminal	Rate-limiting step in synthesis	Vesicle transport	Reuptake	Catabolism	Ionotropic receptors	Metabotropic receptors and associated g protein
Glutamate	Glutamine or α-oxoglutarate	Excitatory amino acid transporter (EAAT)		Vesicular glutamate transporter (VGLUT)	EAAT1–5	Glutamine synthetase	NMDA (NR1, NR2A–D)	Class I (mGluR1&5) $G_{q/11}$
							AMPA (Glu R1–4)	Class II (mGluR2–3) G_i/G_o
							Kainate (Glu R5–7, KA1–2)	Class III (mGluR4, mGluR6–8) G_i/G_o
Glycine	Serine?		Serine trans-hydroxymethylase & D-glycerate dehydrogenase		Glycine transporter (GLYT1–2)		Glycine (α_{1-4}, β subunits)	None
GABA	L-glutamic acid	EAAT	Glutamic acid decarboxylase (GAD_{65} and $_{67}$)	Vesicular GABA transporter (VGAT)	GABA transporters (GATs)	GABA transaminase	$GABA_A$ and $_C$	$GABA_B$ G_i
Acetylcholine	Choline and acetyl CoA	High affinity transporter	Choline transport	Vesicular acetylcholine transporter	High affinity transporter of choline	Acetylcholinesterase (AChE)	Nicotinic AChRs (α_{2-9}, β_{1-4}, γ, Δ subunits)	Muscarinic (M1–5) $G_{q/11}$ – M1, M3, M5 G_i – M2, M4
Dopamine	Tyrosine	Diffusion	Tyrosine hydroxylase (TH)	Vesicular monoamine transporter (VMAT)	Dopamine transporter (DAT)	Monoamine oxidase (MAO) and catecholamine-o-methyltransferase (COMT)	None	D1 and D5 G_s D2, D3, D4 G_i
Norepinephrine and epinephrine	Tyrosine	Diffusion	Tyrosine hydroxylase (TH)	Vesicular monoamine transporter (VMAT)		Monoamine oxidase (MAO) and catecholamine-o-methyltransferase (COMT)	None	α_{1A-D} G_q α_{2A-C} G_i β_{1-3} G_s
Histamine	Histidine	Diffusion	Histidine decarboxylase	Vesicular monoamine transporter (VMAT)	?	Monoamine oxidase (MAO) and catecholamine-o-methyltransferase (COMT)		H_1 G_q H_2 G_s H_3 G_i
5-HT, Serotonin	Tryptophan	Active transport across the blood–brain barrier	Tryptophan-5-hydroxylase	Vesicular monoamine transporter (VMAT)	Specific 5-HT transporter (SERT)	Monoamine oxidase (MAO)	$5\text{-}HT_3$	$5\text{-}HT_{1A-E,5}$ G_i $5HT_2$ G_q $5HT_{4,6,7}$ G_s

down into a number of other metabolites including γ-hydroxybutyric acid (GHB). There is some evidence that the breakdown to GHB is a reversible response and that GHB might be used in the brain to make GABA. GHB has been used in anesthesia and is now an abused street drug; part of its action might be to promote the production of GABA, although a specific GHB receptor is proposed, and GHB is a micromolar (exogenous administration) agonist at the $GABA_B$ receptor and a nanomolar agonist at α4βδ $GABA_A$ receptors.

Once GABA is released into the synaptic cleft its action is terminated mainly by reuptake into the presynaptic terminal using GABA transporters. Some GABA is taken up into surrounding glial cells using the same mechanism but there does not appear to be a mechanism for transfer of this GABA back to the neuron, so the latter must synthesize more GABA to make up for the amount lost. The GABA receptors are divided into an ionotropic group ($GABA_A$, $GABA_C$) and a metabotropic group ($GABA_B$). The $GABA_A$ receptor (Figure 9.3) is a ligand-gated chloride channel and when activated will tend to hyperpolarize the cell. It contains four subunits, and at least 21 proteins (α_{1-7}, β_{1-4}, γ_{1-4}, Δ, ε, θ, ρ_{1-3}) have been identified that can be used to make up this receptor, giving it a great deal of structural diversity. It has been shown that some of these variations have different relative sensitivities to GABA providing the potential for different levels of response to release of the same neurotransmitter. The inhalant anesthetics may activate the $GABA_A$ receptor but this is unlikely to be the only site of action. Propofol and etomidate enhance the action of GABA at the receptor at low concentrations and directly activate the $GABA_A$ receptor at higher concentrations, while barbiturates are much less selective for this receptor. The benzodiazepines have a separate binding site on the receptor that enhances the opening of the channel when natural GABA attaches to its binding site (Figure 9.3). Alfaxalone may affect the GABA receptor by binding to the transmembrane portion of the receptor (Akk et al., 2009). The $GABA_B$ receptor is coupled to G_i proteins that are indirectly linked to potassium channels (increased conductance) and calcium channels (decreased conductance). The latter effect is important for $GABA_B$ receptors located on the presynaptic membrane since this will decrease the amount of calcium released into the cell following an action potential and therefore decrease the release of GABA from the terminal (autoinhibitory response). It is thought that baclofen reduces GABA release via this mechanism.

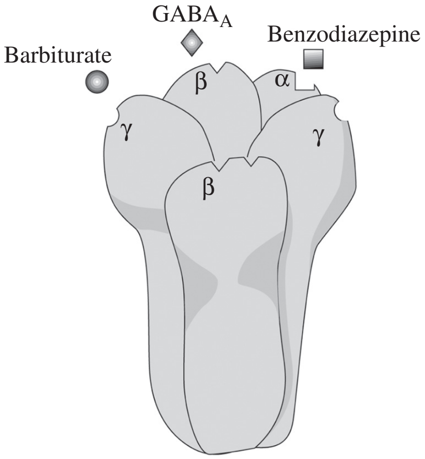

Figure 9.3 $GABA_A$ receptor showing the pentameric structure and protein subtypes that make up the receptor. Neurotransmitters and drugs attach to different subunits on the receptor.

The Amines

Acetycholine: Acetylcholine (ACh) is synthesized from choline and acetyl coenzyme A (acetyl CoA) in a reaction catalyzed by choline acetyltransferase (ChAT). Choline is mainly derived from the blood and from the phospholipid, phosphatidylcholine, and is transported into cells by a high-affinity transporter. Acetyl CoA is derived from glucose or citrate in the mammalian CNS. The synthesized ACh is packed into vesicles via a vesicular acetylcholine transporter. Once released into the synaptic cleft, the ACh attaches to a receptor. In the CNS most of these are nicotinic receptors (nAChRs), another group of pentameric proteins with multiple possible subunits (α_{2-9}, β_{1-4}, γ, Δ). These are nonspecific ion channels that are excitatory. Some areas of the CNS, especially the forebrain and striatum, also contain muscarinic receptors that are metabotropic G protein-coupled receptors (M1–5). Activation of these receptors has an inhibitory effect on dopamine-mediated motor responses. The synaptic cleft also contains acetylcholinesterase (AChE), which is a highly efficient enzyme (5,000 molecules of ACh hydrolyzed/ second/ molecule AChE) that promotes the breakdown of ACh to choline and acetic acid. The choline is taken back up into the nerve terminal for remanufacture to ACh. Atropine acts on central muscarinic receptors but is not specific to any one subtype. Organophosphates block the action of AChE, thus allowing accumulation of ACh in both the PNS and CNS. This leads to typical signs of parasympathetic activation—salivation, lacrimation, urination, diarrhea, and bradycardia – but may also be accompanied by convulsions and eventually coma and death.

Dopamine: Dopamine is synthesized from tyrosine, with tyrosine hydroxylase (TH) catalyzing the conversion of tyrosine to L-DOPA and DOPA decarboxylase catalyzing the conversion to dopamine. The production of an activated phosphorylated TH is dependent on calcium ion concentration, cyclic AMP, and a tetrahydrobiopterin (BH_4). The latter binds to a site on TH that can also bind dopamine, so increasing concentrations of dopamine will inhibit the production of more dopamine. Dopamine is packed into synaptic vesicles by a vesicular monoamine transporter (VMAT) and can then be released into the synapse from these vesicles. Termination of the action of dopamine is mainly by reuptake into presynaptic terminals by a dopamine transporter (DAT). The resorbed dopamine is converted to dihydroxyphenylacetic acid (DOPAC) by monoamine oxidase in the nerve terminal. Dopamine taken up by surrounding glial cells is converted to homovanillic acid by catecholamine-*O*-methyltransferase (COMT). There are species differences in the relative importance of these reactions.

Dopamine receptors can be divided into two forms, D1 and D2-like. Dopamine1 and D5 are classed together and D2–4 are the D2-like receptors. These are all metabotropic G protein-coupled receptors. The D1 receptors are linked to a G_s protein that increases the cellular concentration of cAmp while the D2 receptors are linked to a G_i protein having the opposite effect. D1 receptors are generally found on postsynaptic membranes and while D2 receptors are present on some postsynaptic sites they are more commonly found as autoreceptors on the presynaptic membrane. At this site they seem to be able to affect both synthesis and release of dopamine from the nerve terminal.

Dopamine is found in a number of regions of the brain but its presence in the corpus striatum is thought to play a major role in motor coordination and locomotion. Dopamine is also involved with reward, reinforcement, and motivation, and it is this aspect that contributes to its role in drugs of addiction. For example, cocaine inhibits DAT, thus prolonging the presence of dopamine in the synaptic cleft and prolonging its action on the postsynaptic membrane. Phenothiazines are thought to be dopamine antagonists and hence they reduce motivation and action while monoamine oxidase inhibitors will increase dopamine release and are used in the treatment of depression. Drugs that block D2 receptors will decrease the release of dopamine and at high enough doses will produce catalepsy.

Norepinephrine and epinephrine: These are much less important neurotransmitters within the CNS compared with dopamine (about one-quarter to one-third of the number of neurons containing dopamine) but are also involved in wakefulness, attention, and feeding behavior. These neurotransmitters play a dominant role in autonomic nervous system function, and were fully described in Chapter 7. Norepinephrine is produced from dopamine with the aid of dopamine-β hydroxylase and epinephrine is further synthesized with the aid of phenylethanolamine-*N*-methyltransferase. The latter is found in a discrete number of neurons that are different from those secreting norepinephrine. Both neurotransmitters are packed into vesicles using VMAT and are metabolized by monoamine oxidase and COMT when taken back up into the cell. Norepinephrine is removed from the synaptic cleft by norepinephrine transporter (NET) and this will also transport epinephrine – no specific epinephrine transporter has been identified.

The α- and β-adrenergic receptors for these neurotransmitters are G protein-coupled metabotropic receptors and are further divided into α_{1A-D}, α_{2A-C}, and β_{1-3}. The α_{1A-D} are associated with a G_q protein that results in a slow depolarization due to the inhibition of K^+ channels while the α_{2A-C} receptors are associated with a G_i protein that results in hyperpolarization. The α_2 receptors are found on both presynaptic and postsynaptic terminals. They act as autoreceptors on the presynaptic terminal to decrease the release of norepinephrine. Since wakefulness is dependent on tonic activity on these neurons it follows that the inhibition of this activity would tend to lead to sedation and lack of movement. This is the principle behind the use of α_2 agonists, which decrease the release of norepinephrine and may hyperpolarize the postsynaptic cells as well. α_2 antagonists have the opposite effect and can cause central excitation when administered alone.

Histamine: Histamine is produced from the amino acid histidine, catalyzed by the enzyme histidine decarboxylase. It is transported into vesicles using VMAT and released into the synaptic cleft where it can act on one of three histamine receptors (H_{1-3}). Chapter 19 should be consulted on histamine's role outside of the CNS. No plasma membrane transporter has been identified for histamine but it is metabolized by histamine methyltransferase and monoamine oxidase. The histamine receptors are G protein-coupled metabotropic receptors. H1 is coupled with a G_q protein affecting inositol phospholipase, H2 is coupled with a G_s protein that increases the cAmp concentrations, while the H3 receptor is coupled with a G_i protein with opposing effects. It is thought that the H3 receptor is the autoreceptor on the presynaptic terminal involved in decreasing the release of histamine and possibly other neurotransmitters present in the same cells.

Histamine is also thought to be involved in aspects of wakefulness and is strongly associated with the vestibular apparatus, explaining the use of antihistamines in the control of motion sickness and that drowsiness is a common effect with antihistamines that cross the blood–brain barrier.

5-Hydroxytryptamine or serotonin: 5-Hydroxytryptamine (5-HT) is synthesized from tryptophan via 5-hydroxytryptophan catalyzed by the enzymes tryptophan-5-hydroxylase and then aromatic L-amino acid decarboxylase. Tryptophan is transported into the brain via an active process that also transports other large neutral amino acids. This means that the production of 5-HT is dependent not only on the concentration of tryptophan in the diet but also on the relative amounts of tryptophan. Dietary restriction of tryptophan depletes 5-HT production while increased dietary tryptophan increases 5-HT production up to a point where the tryptophan-5-hydroxylase enzyme system becomes saturated. As with many biological systems a treatment that alters one component may be compensated for by changes in another component. For example, increased dietary tryptophan could be compensated for by a decrease in tryptophan-5-hydroxylase. Once synthesized 5-HT is packed into vesicles using VMAT and once released, it is transported back into the cell by a specific 5-HT transporter (SERT). This protein is the target for a number of therapeutic agents called the *selective serotonin reuptake inhibitors (SSRIs)*, of which fluoxetine (Prozac) is one. Once taken up, 5-HT is mainly broken down using monoamine oxidase.

At least 12 5-HT receptors have been identified (5-HT$_{1A-E}$, 5-HT$_{2A-C}$, 5-HT$_{3-7}$), with the majority being G protein-coupled metabotropic receptors. The exception to this is 5-HT$_3$, which is a ligand-gated ionotropic receptor that allows influx of cations to cause excitation of the postsynaptic membrane. The 5-HT$_1$ receptors are coupled with G$_i$ proteins and 5-HT$_2$ with G$_q$ proteins, and the others are thought to be associated with G$_s$ proteins (with the exception of 5-HT$_5$, which is probably associated with a G$_i$ protein).

5-HT is also involved in the regulation of sleep and attention and it plays a role in nociception and the control of emesis. Some potent antiemetics such as ondansetron and granisetron are 5-HT$_3$ antagonists. The 5-HT$_3$ receptor may be involved in the mechanism of action of inhaled anesthetics (Solt et al., 2005; Stevens et al., 2005). Serotonin syndrome is a condition described in people that presents with multiple signs, including alterations in conscious state (agitation, anxiety, seizures to lethargy, and even coma), autonomic dysfunction (central and peripheral actions of 5-HT hyperthermia, sweating, tachycardia, hypertension, dyspnea, dilated pupils), and neuromuscular changes (myoclonia, hyperreflexia, and ataxia). This syndrome is being reported more commonly because of the many drugs being prescribed that affect the 5-HT system. Amitriptyline, tramadol, meperidine, and St John's wort all reduce 5-HT uptake, while selegiline and St John's wort reduce 5-HT catabolism. Combinations of these drugs or excessive doses of a single agent can lead to this syndrome (Jones and Story, 2005). In dogs a similar syndrome has been described following ingestion of 5-hydroxytryptophan (Gwaltney-Brant et al., 2000).

Peptides

Many peptides have been identified as playing a part in neurosignaling. These substances are synthesized in the cell body as a prepropeptide that may contain a number of different peptide molecules. Proopiomelanocortin (POMC) is a good example since it contains ACTH, α- and γ-melanocyte stimulating hormone (MSH), γ-lipotropic hormone, and β-endorphin. The final peptide neurotransmitter is generated by peptidases cleaving the prepropeptide into its component parts and this cleavage may be tissue specific. In the anterior lobe of the pituitary, POMC is converted mainly to ACTH, whereas the output from the intermediate lobe consists mainly of β-endorphin and MSH. Most of the peptides are colocalized in synaptic terminals with smaller molecule neurotransmitters (Table 9.2) and their release may be dependent on a different signal strength than with the smaller molecules. Typically, it takes a greater amount of calcium in the synaptic vesicle to get the peptide to be released. Once the peptide has been released it attaches to receptors on the postsynaptic membrane and it generally takes lower concentrations of these molecules, compared with the smaller neurotransmitters, to have an effect. A peptide may also diffuse more broadly to influence neurons further away as suggested by the fact that receptors for some peptides are in separate anatomical locations to the neurons producing the peptidergic agonist. As for the other neurotransmitters, peptide receptors often come in a variety of subtypes and these may be found in different locations of the CNS and subserve different signaling purposes. They are all G protein metabotropic receptors. Once released into the synaptic cleft the peptides are

Table 9.2 Colocalization of some small molecule neurotransmitters with peptides

Neurotransmitter	Peptides found in the same terminals
Glutamate	Substance P Orexin Enkephalin
γ-amino-butyric acid (GABA)	Somatostatin Cholecystokinin (CCK)
Acetylcholine	Vasoactive intestinal peptide (VIP) 5-Hydroxytryptamine (5-HT)
Dopamine	CCK Neurotensin
Norepinephrine	Somatostatin Enkephalin Neuropeptide Y (NPY) Neurotensin
Epinephrine	NPY Neurotensin
5-HT	Substance P Thyrotropin releasing hormone (TRH) Enkephalin

Table 9.3 Peptide neurotransmitters

Neuropeptide	Receptors
Gut/brain peptides	
Substance P, Neurokinin A and B	NK$_{1-3}$
Vasoactive intestinal peptide (VIP)	VPAC$_{1,2}$, PAC$_1$
Neuropeptide Y	Y$_{1-5}$
Cholecystokinin (CCK)	CCK$_{1,2}$
Orexin A and B	OX$_{1,2}$
Pituitary peptides	
Vasopressin	V$_{1,2}$
Oxytocin	OT
Adrenocorticotrophic hormone (ACTH)	MC1–5R
α-melanocyte stimulating hormone (α-MSH)	MC1–5R
Prolactin	
Hypothalamic releasing peptides	
Thyrotropin releasing hormone (TRH)	TRH$_{1,2}$
Luteinizing hormone-releasing hormone (LHRH)	
Somatostatin	SST$_{1-5}$
Growth hormone releasing hormone (GHRH)	
Gonadotropin releasing hormone (GnRH)	GnRHR
Opioid peptides	
Leu-enkephalin	MOP, DOP
Met-enkephalin	MOP, DOP
Endorphin	MOP, DOP
Dynorphin	MOP, KOP
α and β4 neoendorphin	MOP, KOP, DOP
Orphanin FQ	NOP
Others	
Angiotensin	AT$_{1,2}$
Neurotensin	NTS$_{1,2}$
Corticotropin releasing factor (CRF)	CRF$_{1,2}$
Calcitonin gene-related peptide (CGRP)	CGRP$_1$, AM$_{1,2}$
Bradykinin	B$_{1,2}$

catabolized by peptidases that are present in the extracellular matrix.

The peptides can be divided into several groups, which have more to do with their discovery than their function within the CNS: the gut/brain peptides, the pituitary hormones, the hypothalamic releasing factors, the opioid peptides, and others (Table 9.3). Of these, the tachykinins (specifically substance P) and the opioids are the most important with regard to nociception. Substance P is colocalized with glutamate in many of the small fiber afferents that are involved with sensing hot/cold and noxious stimulation and it attaches to one of the neurokinin receptors (NK$_{1-3}$). Opioid peptides (β-endorphin, dynorphin, leu-enkephalin, met-enkephalin, α and β neoendorphin, orphanin FQ) act at the mu, kappa, delta, or orphanin FQ receptors (MOP, KOP, DOP, and NOP). These peptides are present in the dorsal horn of the spinal cord and throughout the CNS, and are largely involved in the modulation of nociception. The relative abundance and location of these peptides and their receptors varies between species.

Other Neurotransmitters

Purines: ATP, adenosine, and adenine dinucleotides are released into the synaptic cleft of some neurons. These differ from classic neurotransmitters in that ATP and adenine dinucleotides may be in the same synaptic vesicle as other small molecule neurotransmitters, while adenosine is not stored in vesicles. Further ATP can be converted to adenosine in the synaptic cleft to act on its receptors separately from the ATP. Adenosine attaches to P$_1$ receptors (A$_1$, A$_{2A-B}$, A$_3$), which are all G protein-coupled receptors; ATP can attach to P$_{2X1-5}$ (ionotropic) or P$_{2Y1,2,4,6,11}$ (metabotropic) receptors. Adenosine has hypnotic and anxiolytic effects and is involved in the modulation of nociception.

Endocannabinoids: Anandamide and 2-arachidonylglycerol (2-AG) are thought to act on cannabinoid receptors (CB1-2). This is an unusual signaling pathway because the ligands are released from the postsynaptic cell and have an effect on the presynaptic terminal. The current hypothesis is that the increase in postsynaptic cytoplasmic calcium, triggered by depolarization, stimulates the release of the cannabinoid neurotransmitter and this attaches to CB1 receptors on the presynaptic terminal resulting in an inhibitory effect. The CB1 receptors are located on GABA terminals so the inhibition of GABA release reduces the inhibitory effect of GABA and causes stimulation (e.g., increased appetite associated with cannabis administration).

Agmatine: Agmatine is a decarboxylated arginine that has been found as an endogenous neurotransmitter and has been proposed as a natural agonist at the imidazoline receptor. These receptors are of interest to the clinician because many of the α$_2$ agonists used in practice are imidazolines (e.g., medetomidine, detomidine, romifidine). However, investigations with this substance have also shown that it interacts with the glutmatergic system and acts as an antagonist at the NMDA receptor and inhibits nitric oxide synthase. Given its colocation in multiple sites it is thought to be more of a cotransmitter rather than the primary imidazoline agonist. Agmatine has been shown to be able to decrease pain resulting from inflammation, neuropathy, and spinal cord injury (Fairbanks et al., 2000) and may have many other therapeutic effects (Piletz et al., 2013).

Role of Nonneuronal Cells

The normal development and activity of the CNS is dependent on the glial cells. The three types of glial cells – oligodendrocytes, astrocytes, and microglia – are all intimately associated with neurons. The oligodendrocytes form myelin sheaths for neurons in the CNS (equivalent to the Schwann cells in the PNS), the astrocytes ensheath synaptic junctions, provide a scaffolding within the CNS, and ensheath the capillaries as part of the blood–brain barrier. Microglia are present throughout the CNS and were thought to be relatively quiescent until needed to provide clean-up duties by engulfing foreign materials and pathogens. It is now clear that all of these cells also have a function in cell-to-cell signaling even though they do not have the ability to depolarize. As indicated above in this section, astrocytes that surround the synaptic junctions play a role in absorbing neurotransmitters and, in some cases, metabolizing them and/or transferring them back to the presynaptic terminal. Some of these molecules may also activate the glial cells – substance P, ATP, and excitatory amino acids have all been shown to do this. Other substances that may be released from the neuron to activate the glia include nitric oxide and fractalkine. Disturbingly, it has also been shown that morphine can activate glial cells and so the administration of an opioid analgesic may be enhancing the transmission of nociceptive information (Watkins et al., 2005)! However, the depolarization of the synaptic terminal itself may also activate the astrocyte and open calcium channels. An influx of calcium into the astrocyte may induce increased production of factors that can feed back to the synapse or this change may be transmitted to other astrocytes via the tight junctions that connect these cells. Factors released by glia that might alter synaptic transmission include prostaglandins, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), the neurotrophins NT-3 and NT-4/5, and the proinflammatory cytokines interleukin-1 (IL-1) and tumor necrosis factor (TNF). As an example, it has been shown that BDNF activates a specific sodium channel on some neurons and this plays a role in long-term potentiation. Thus the glial cells may release factors into their local environment that affect the activity of a number of neurons, adding an amplifying effect to signals being processed through this network. Using drugs that modify the activation of glial cells (e.g., minocycline) or drugs that block cell signaling pathways internally (e.g., p38 MAP kinase inhibitors) or the production of activators (e.g., antiprostaglandins) has the potential to alter pain perception.

Interactions Between Neurotransmitter Systems

The descriptions above are overly simplistic in many areas since there are multiple interactions between the neurotransmitters and the processes that control them. The colocation of neurotransmitters within the same presynaptic terminals means that even the signal produced from a single terminal can be modified by the intensity of the incoming signal or other chemicals in the local environment. It is also being shown that receptor proteins can interact directly to modify the signal being received. For example, D5 interacting with $GABA_A$ and D1 with NMDA. In the latter case activation of D1 receptors decreases the cell-surface localization of NMDA receptors, whereas activation of the NMDA receptor increases the number of D1 receptors on the cell surface (Lee and Liu, 2004).

Blood–Brain Barrier

In order for any drug to produce an effect on the CNS it has to gain access to the neurons and other cells in the brain. Neuronal function relies on having a very stable extracellular ionic content and limiting the number of “foreign” substances to a minimum, such that exposure of neurons to nanomolar concentrations of some neurotransmitters can produce an effect. In order to achieve this, animals have developed a barrier from the blood that allows the passage of small molecules but restricts the passage of ionic compounds and most larger molecules (Abbott, 2005; Hawkins and Davis, 2005). This barrier is formed by tight junctions between endothelial cells, pericytes, a basement membrane, and by astrocytes that encase the capillaries (Figure 9.4). As well as the tight junctions the endothelial cells have an increased content of mitochondria, a lack of fenestrations, and minimal pinocytotic activity compared with capillaries in other parts of the body. The barrier is not present in some areas of the brain, namely, the circumventricular organs (CVOs). These include the subfornical organ at the roof of the third ventricle, the median eminence and the organum vasculosum of the lamina terminalis in the floor of the third ventricle, the pineal gland and the subcommissural organ at the back of the third ventricle, and the area postrema near the fourth ventricle. The chemoreceptor trigger zone (CTZ) is in the area postrema and its lack of a blood–brain barrier (BBB) may be part of the explanation for the rapid onset of emesis with some opioids (e.g., morphine). These drugs produce an antiemetic effect at the vomiting center, which is behind the BBB, and so the resultant effect is dependent on the rate at which the drug activates these two areas. With hydrophilic drugs, penetration to the vomiting center is slower than the effect on the CTZ with resulting emesis. More lipophilic opioids (e.g., fentanyl) inhibit the vomiting center at about the same time as they excite the CTZ, giving a much lower incidence of emesis. These CVOs have a comparatively small surface area and are thought to be separated from the rest of the brain by a glial barrier, thus limiting the spread of material taken up through the CVOs. The choroid plexus is comparatively leaky, but substances that gain access to the CSF have relatively limited access to neuronal tissue. The

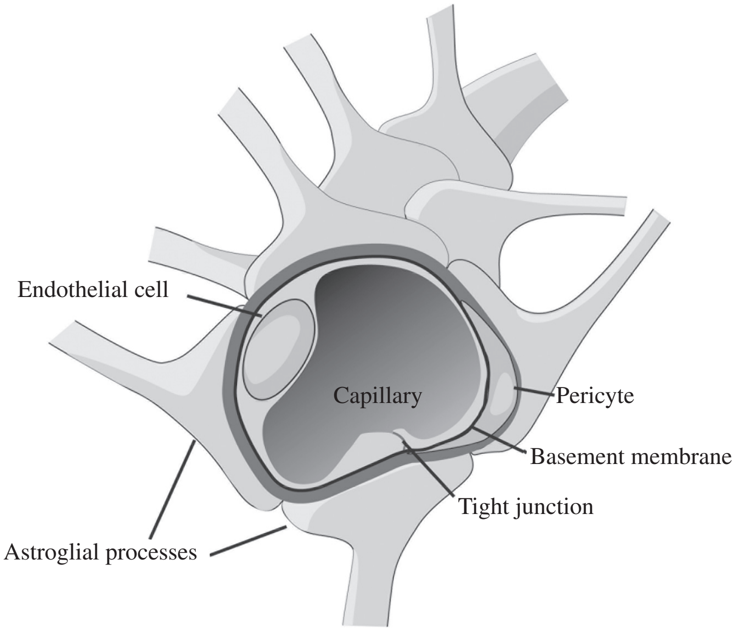

Figure 9.4 Blood–brain barrier. The endothelial cells have tight junctions that link the cells in such a way as to minimize passage of material through the cells. These cells also have fewer pinocytotic vesicles and more mitochondria than capillary endothelial cells elsewhere in the body. These cells are surrounded by a basement membrane and there are pericytes in this location. Astroglial foot processes surround the outside of the capillary.

surface area of the choroid plexus is about 1/1000th of the area of the BBB.

The transfer of molecules across the BBB under normal physiological conditions is regulated by three factors. There are solute carrier (SLCs) proteins that are used to carry essential molecules such as glucose, amino acids, amines, adenine, adenosine, vitamins, and thyroid hormones across the barrier. Over 350 of these SLCs have been identified and they are referred to using a number and letter system (e.g., SLC7A5, SLC47A2). There are receptor-mediated methods involved in the transcytosis of some peptides. Insulin, transferrin, and leptin are examples of peptides that are transferred by receptor-mediated transcytosis. There are also ATP-binding cassette (ABC) transporters that move substances in both directions across the BBB. There are seven subfamilies of these ABC transporters and they play a role in many tissues in the body, as introduced in Chapter 2. The seven subfamilies are designated by letters (e.g., ABCA1, ABCB2, ABCG2). The ABC transporters move substances in both directions depending on their location on the luminal side of the endothelial cell (ejecting substance) or the abluminal side of the cell, where they may be part of an active transportation of molecules into the brain. The best studied are the active efflux systems that are designed to eject unwanted metabolites from the extracellular space in the brain and have now been shown to have significant effects on the access of some drugs to the CNS. One of the proteins involved in this process is ABCB1 or P-glycoprotein, which has a wide substrate specificity and transports a range of drugs from the CNS. This has become of clinical interest with the discovery of the *MDR-1* gene that is responsible for the production of ABCB1. In some herding breed dogs (Collies and Australian Shepherds) a polymorphism of this gene results in increased toxicity to a number of drugs that was first recognized as ivermectin toxicity (Mealey, 2004). It is now evident that a lack of ABCB1 may also alter the brain concentration of a number of other drugs. In *MDR-1* deficient mice, brain concentrations of morphine and fentanyl were increased by 25% and the uptake of methadone was increased by 200–1500% (Dagenais et al., 2004; Wang et al., 2004). Butorphanol may have a more profound effect in MDR-1 deficient dogs. Phenothiazines have been shown to have an inhibitory effect on P-glycoprotein and may thus alter the uptake of other drugs into the brain. Acepromazine seems to have a more profound sedative effect in these dogs (Mealey, 2006). The pharmacogenomics of this phenomenon is discussed in Chapter 50.

The rate at which drugs cross the BBB is governed by the above activities and also the physicochemical properties of the drug itself. Molecular size, lipid solubility, hydrogen bond donor sites, and external oxygen and nitrogen affect the passage across the BBB. Polar molecules are not able to cross the BBB, so the drug needs to be present in an unionized form in order to get into the brain. Lipid solubility is measured in a number of ways but one of the most common is to measure the octanol/buffer partition coefficient and express this as a log value, usually given as Log P. Molecules that are less likely to penetrate the BBB would have a molecular weight >450 g/mol, a Log P >4, >5 hydrogen bond donors, and the sum of nitrogen and oxygen atoms >10. On the other end of the scale, molecules with low Log P values (<2) also have low penetrance, leading to a sigmoid-shaped curve when Log P is plotted against brain uptake. This can be illustrated by the pharmacodynamics of morphine (Log P = 0.1), alfentanil (Log P = 2.1), and sufentanil (Log P = 3.24), where the peak effects of the drugs are approximately at 2–3 hours, 1 minute, and 6 minutes, respectively (Lotsch, 2005).

Epidural Barrier

Drugs administered into the epidural space must diffuse through the dural membranes in order to reach the spinal cord where they might have an effect (Bernards and Hill, 1990, 1991). This diffusion is dependent on many of the usual factors (concentration gradient, molecular size, polarity, lipid solubility, and distance) but the same sigmoid effect of lipid solubility is seen here as well (Bernards and Hill, 1992). This is demonstrated in Figure 9.5 where Log P is plotted against meningeal permeability measured in vitro. Epidural fat plays a role in that very lipid-soluble drugs tend to get absorbed here thus limiting their uptake into the spinal cord. The main meningeal barrier to diffusion is the pia–arachnoid membrane with its complex mix of aqueous fluids (CSF and extracellular fluid) and lipid membranes. The distance effect is illustrated in Figure 9.6 where the time to onset is plotted against depth across a number of species following intrathecal administration (Yaksh, 2005).

Principles of Anesthesiology

Anesthesiology is defined as the art and science of administration of anesthesia. The term also describes a clinical specialty of medicine (including veterinary medicine) that emerged during the early 1900s when a few physicians began devoting full time to the clinical administration of anesthetics. Anesthesiology was officially recognized as an organized specialty in medicine with the establishment in 1938 of a peer-certifying body of physicians, the American Board of Anesthesiologists (ABA). Initially, the ABA was an affiliate board of the American Board of Surgery but in 1941 it became an independent board. In addition, in 1940, a section of anesthesiology was formed within the American Medical Association. In 1975, the American College of Veterinary Anesthesiologists was officially recognized by the American Veterinary Medical Association as the body to certify veterinarians as specialists in veterinary anesthesia. Broader summaries of the development of anesthesiology are available elsewhere (Smithcors, 1971; Weaver, 1988; Larson, 2005; Weaver and Hall, 2005; Steffey, 2014). The central role of the anesthetist is: (i) to apply methods to minimize or eliminate pain, relax muscles, and facilitate patient restraint during surgical, obstetrical, and other medical, diagnostic, and therapeutic procedures; and (ii) to monitor and support life functions in patients during the operative period as well as in critically ill, injured, or otherwise seriously ill patients. The skills and knowledge that have developed in the field have extended the clinical practice of anesthesiology into intensive care, cardiac and pulmonary resuscitation, and the control of pain problems unrelated to surgery. The word *anesthesia* is derived from the Greek for “insensible” or “without feeling.” The word does not necessarily imply loss of consciousness.

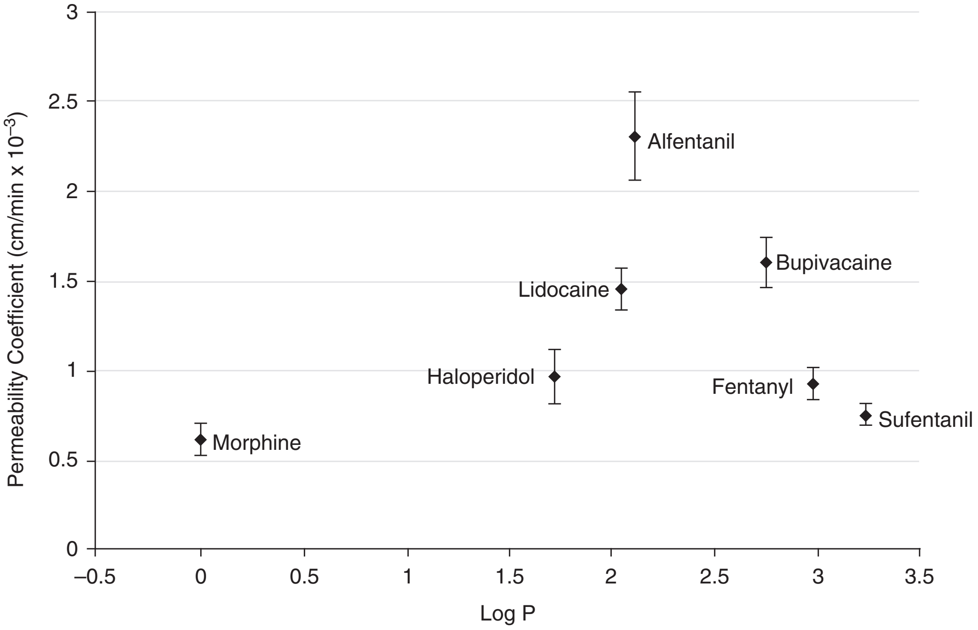

Figure 9.5 Permeability of dural membranes to various drugs used in anesthesia versus their octano: buffer partition coefficient, expressed here as Log P. Source: Data from Bernards and Hill, 1992.

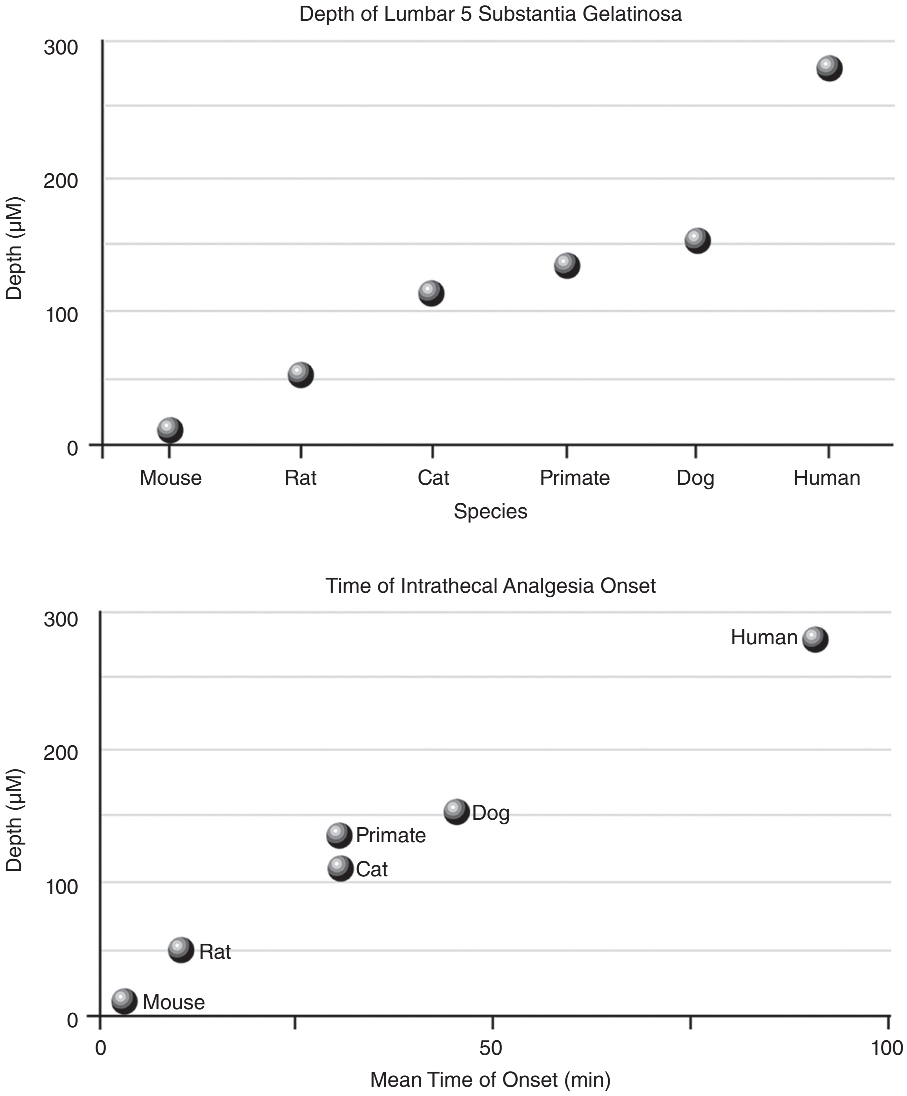

Figure 9.6 Relationship of the distance from the surface of the spine to the substantia gelatinosa at the fifth lumbar segment in various species (top) and the rate of onset of morphine (intrathecal at minimally effective doses) in these different species (bottom). Source: Drawn from Yaksh TL, Spinal Analgesic Mechanisms. In *Pain 2005—An Updated Review.* International Association for the Study of Pain, 909 NE 43rd St, Suite 306, Seattle, WA 98105-6020.

In the realm of veterinary anesthesiology, anesthesia and anesthetics are used for a variety of reasons (Table 9.4).

Anesthetic Use

Perception of Noxious Stimuli (Pain)

Prevention of the perception of a noxious stimulus during surgery is the primary justification for anesthesia. A noxious stimulus is defined as a stimulus that is potentially damaging to body tissue. Nociception has no emotional or perceptional connotation. Pain is an unpleasant sensory and emotional experience; it is a perception, not a physical entity. The perception of pain depends on a functioning cerebral cortex. The concept of pain includes several interdependent dimensions: the sensory/discriminative and motivational/affective.

Table 9.4 Use of anesthetics in animals

- Elimination of sensitivity to noxious stimuli
- Humane restraint (e.g., protect animal, facilitate diagnostic or surgical procedure)
- Technical efficiency (e.g., protect personnel, facilitate diagnostic or surgical procedure)
- Specific biomedical research tool (e.g., sleep time)
- Control convulsions
- Euthanasia

It is not the intent to sidetrack here into semantic issues or to belabor what are, to some, fine points but it is necessary for completeness to stress that contemporary reasoning holds that pain is a subjective response in conscious human beings. When considering “pain” in animals, it is important to recognize that our knowledge of pain in animals is largely inferential. We approach the subject with “the tacit assumption…that stimuli are noxious and strong enough to give rise to the perception of pain in animals if the stimuli are detected as pain by human beings, if they at least approach or exceed tissue damaging proportion and if they produce escape behavior in animals" (Kitchell and Erickson, 1983).

Classical approach to mechanisms of pain: Before further discussion of methods by which insensitivity is produced, it is helpful to briefly review the mechanisms whereby an individual becomes aware of and reacts to a noxious stimulus (Figure 9.7). This inclusion here is justified on the basis that knowledge of these mechanisms offers the clinician targets for single or multiple attacks in an attempt to abolish or minimize pain.

Acute pain that is provoked by disease or injury (planned or unplanned) is the net effect of many interacting and complex anatomic paths and physiological mechanisms. The stimulus excites a specialized receptor organ, the nociceptor. Nociceptors are distributed throughout the body but are frequently grouped as somatic (cutaneous, muscle, bone, joint, fascia) or visceral. Nociceptors are located at the termination of free nerve endings of small poorly myelinated or nonmyelinated A-delta and C afferent nerves. The nociceptors transduce the stimuli into nociceptive impulses that are transmitted to the CNS (see Section Transduction). Impulses that originate from areas below the head are transmitted via fibers that synapse with interneurons or second-order neurons in the dorsal horn of the spinal cord. Impulses from the head travel via fibers within the cranial nerves to the medulla, where they synapse with neurons in the trigeminal nuclei (medullary dorsal horn). In the spinal cord, the signal is subjected to a variety of potential modulating influences in the dorsal horn. For a long time the dorsal horn was considered to perform simply as a relay station. More recent evidence indicates it contains an incredibly complex circuitry and rich biochemistry that permits not only reception and transmission of nociceptive impulses but also a large degree of signal processing. After being subjected to these modulating influences, some of the impulses may then stimulate somatomotor and preganglionic sympathetic neurons and provoke nocifensive reflex responses. Nociceptive impulses also activate other neurons making up the ascending systems that pass to the brain stem and brain. Supraspinal systems that are probably involved in processing nociceptive information to progressively higher levels of awareness include the reticular formation, limbic system, hypothalamus, thalamus, and cortex.

Activation of the reticular formation results in abrupt awakening, diffuse alertness, and initiation of protective homeostatic responses. In turn, affective (emotional) alertness is obtained through cortical arousal. The animal is now fully knowledgeable regarding the cause and strength of the noxious stimulus and its relationship to the environment. The animal in turn reacts with a coordinated response.

The above simplified description encompasses the processes associated with acute pain that might be caused by a surgical incision into normal tissue. However, chronic pain states can involve changes in activity at any part of the nociceptive system. As indicated above, the inflammatory mediators released into the tissues will sensitize the nociceptors and may lead to alteration in the receptors expressed (e.g., TRPV-1). In the spinal cord a barrage of input from the nociceptors can lead to changes in the glutamate receptors with activation of the NMDA receptors and an enhancement of the incoming signals. In addition to this, the glial cells may also be activated

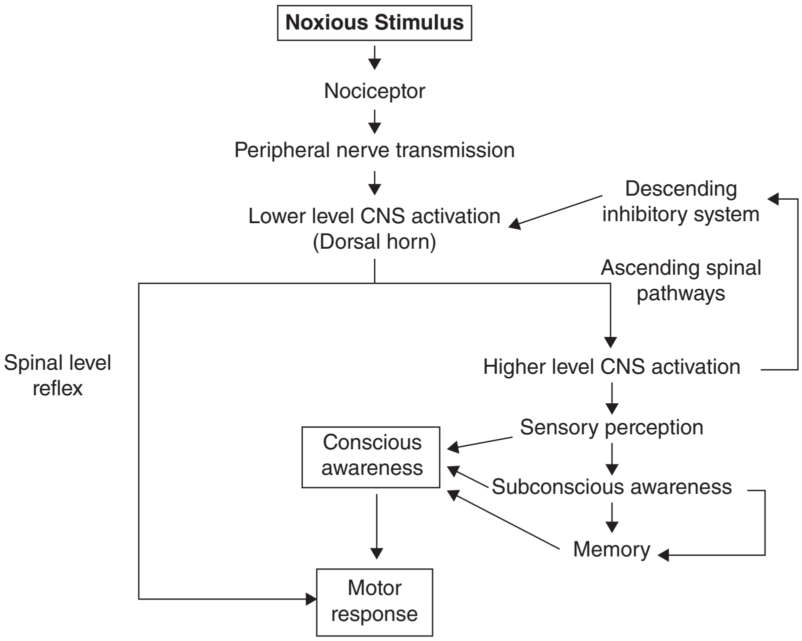
Noxious Stimulus
Nociceptor
Peripheral nerve transmission
Lower level CNS activation (Dorsal horn)
Descending inhibitory system
Ascending spinal pathways
Spinal level reflex
Higher level CNS activation
Sensory perception
Conscious awareness
Subconscious awareness
Memory
Motor response

Figure 9.7 Events in the reaction to a noxious stimulus.

and both amplify the signal and sensitize the neurons to further input. It is thought that phenomena such as phantom limb pain and the chronic pain associated with interstitial cystitis are largely mediated by changes in the dorsal horn. The central processing of pain in the brain also provides on-going modifiers to the incoming signals. It is well established that the system that provides descending inhibition of nociception can be so effective that it can prevent the animal from feeling pain associated with acute injury but the effect on chronic pain is less understood. Stress can stimulate both the descending inhibitory and facilitatory pathways and it seems that acute stress may increase inhibition whereas chronic stress facilitates nociceptive input. These elaborate mechanisms allow us to use drugs in chronic pain that may not be analgesics in their own right but may affect part of these neurochemical events to decrease pain. Examples include minocycline to block glial activation and gabapentin and tricyclic antidepressants to decrease descending facilitation.

For more detailed information the reader is referred elsewhere (Fishman et al., 2010; Fox, 2010; McMahon and Kolzenburg, 2013).

Immobility

Although the primary reason for anesthetic delivery is to render the animal insensible to pain, restraint and technical efficiency are also long-recognized important considerations. Although viewed as an extreme in approach today, Alexandre Liautord, a Frenchman, noted in his 1892 *Manual of Operative Veterinary Surgery:* "In veterinary surgery, the indication for anesthesia has not, to the same extent as in human, the avoidance of pain in the patient for its object, and though the duties of the veterinarian include that of avoiding the infliction of *unnecessary* pain as much as possible, the administration of anesthetic compounds aims principally to facilitate the performance of the operation for its own sake, by depriving the patient of the power of obstructing, and perhaps even frustrating its execution, to his own detriment, by the violence of his struggles, and the persistency of his resistance. To prevent these, with their disastrous consequences, is the prime motive in the induction of the anesthetic state" (quoted in Smithcors, 1971). More in keeping with contemporary thought are the words of George H. Dadd written in 1854 in *The Modern Horse Doctor:* "We recommend that, in all operations of this kind, the subject be etherized, not only in view of preventing pain, but that we may, in the absence of all struggling on the part of our patient, perform the operation satisfactorily, and in much less time after etherization has taken place than otherwise. So soon as the patient is under the influence of that valuable agent, we have nothing to fear from his struggles, provided we have the assistance of one experienced to administer it" (quoted in Smithcors, 1971). Many of these same clinical principles can and should also be applied directly to the research environment.

Table 9.5 Routes by which anesthetic or anesthetic adjuvant drugs are administered in animals

1. Topical
    a) Cutaneous
    b) Mucous membrane
2. Injection
    a) Intravenous
    b) Subcutaneous
    c) Intramuscular
    d) Intraperitoneal
    e) Intraosseous
3. Gastrointestinal tract
    a) Oral
    b) Rectal
4. Respiratory system (e.g., inhalation)

Anesthesia Classified

Anesthesia is produced by both chemical (i.e., drugs) and physical (e.g., sensory nerve destruction) means. Anesthetic drugs are frequently classified according to their route of administration (Table 9.5). Some drugs are only suited for common delivery via one route (e.g., the inhalation anesthetics), while many of the injectable agents may be administered in a variety of different manners depending on the drug and the desired endpoint of effect.

Local and Regional Anesthesia

Anesthetics are also classified according to the region of the body influenced (Table 9.6). For example, a local anesthetic is administered to desensitize a localized or regional area of the body. It is deposited in close proximity to a nerve membrane, causing nerve conduction

Table 9.6 Techniques of anesthesia based on extent of loss of sensation

1. Local/regional: drugs placed in close proximity to nerve membranes, causing conduction block
    a) Topical or surface
    b) Area infiltration
        i. Subdermal
        ii. Intravenous regional
    c) Perineural (i.e., nerve trunk)
    d) Peridural (i.e., epidural or caudal)
    e) Subarachnoid (i.e., spinal)
2. General anesthesia: state of controlled, reversible CNS depression (including unconsciousness) produced by one or multiple drugs
    a) Injectable
    b) Inhalation
    c) Balanced

blockade. This may be used in the conscious patient and is widely used in large animal practice to allow procedures to be done in the standing patient. Increasingly, local anesthetics are being used in conjunction with general anesthesia or heavy sedation to block the nociceptive input from the surgical site.

General Anesthesia

General anesthesia is a condition induced by pharmacological or other means that results in controlled, reversible CNS depression. It is true that some drugs in the process of producing anesthesia cause excessive stimulation and activity in the brain, but all anesthetic agents ultimately reduce and stop electrical activity in the brain and decrease brain oxygen consumption. On this basis, it is proper to characterize anesthetic agents as CNS depressants.

There is no consensus about the essential elements that comprise the behavioral state that we term *general anesthesia*. Further complication is added by trying to match human and animal studies. For example, sedation is sometimes defined as loss of appropriate responsiveness to verbal command – relatively easy for use with human subjects, more difficult or not possible with animals. For this introductory overview and further discussion, basic elements of general anesthesia will, in addition to reversibility, include absence of awareness (unconsciousness), no recall of events at the conscious level (amnesia), conscious insensitivity to pain (analgesia), and muscle relaxation and diminished motor response to noxious stimulation (immobility). The importance of minimal autonomic nervous system response to noxious stimulation has also been emphasized by some in clinical applications.

General anesthesia has traditionally been considered a dose-related continuum of a series of events passing into each other (Figure 9.8), from alert wakefulness through lethargy and drowsiness (sedation), unconsciousness (with and without somatic and visceral response to external stimuli), coma, and death. The wakefulness to coma series implies progressive loss of higher CNS (cortical) function followed by depression of brain stem functions. While portions of this scheme have been challenged, its use remains, in general, acceptable and offers a convenient method to set the stage for further discussion of introductory level understanding or principles of general anesthesia.

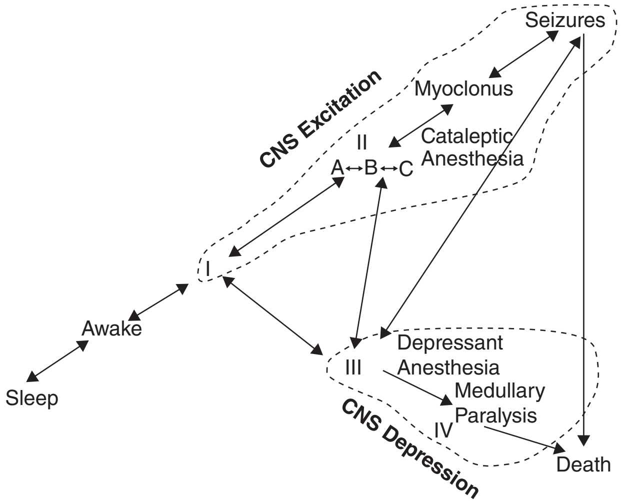

Figure 9.8 Schematic representation of the stages of anesthesia according to Winters et al. (1972) and Winters (1976). The schematic has been modified slightly from its original description. I–IV refer to the classic stages of anesthesia.

Techniques of general anesthesia: General anesthesia is pharmacologically induced and maintained in animals via one of two general methods. The oldest approach, which is still used in certain animal applications, is the single-agent technique. With this technique, an agent such as pentobarbital, thiopental, or ketamine is administered at sufficient dose to provide the complete spectrum of characteristics of general anesthesia. This method is simple but may be more life threatening, especially under adverse circumstances, including animal ill health. Because *all* anesthetic agents have some undesirable effects when used alone (the LD_{50}/ED_{50} is commonly no more than 2–4), modern anesthetic practice most commonly involves the use of combinations of drugs. With more modern technique, multiple drugs are used, at lower doses than would be needed if used alone, with each drug being used for a specific purpose (amnesia, immobility, analgesia, unconsciousness). This technique is known as *balanced anesthesia* and was originally conceived to encompass techniques that used a drug for each component. This might have been an inhalant for unconsciousness, a benzodiazepine for amnesia, an opioid for analgesia, and a neuromuscular blocking drug for immobility. In modern usage *balanced anesthesia* is often applied to any technique using more than one class of drug as part of an anesthetic technique. The ultimate intent is to take advantage of the desirable features of selected drugs while minimizing their potential for harmful depression of homeostatic mechanisms. This technique is especially advantageous when used with physiologically compromised individuals. Another disadvantage of using single drugs for anesthesia is that they may provide unconsciousness and immobility but may leave the nociceptive system intact such that the surgical procedure will still enhance central facilitation of nociceptive input. This sets the animal up for a worse pain experience in the postoperative period and possibly long-term pain. Although this phenomenon was recognized in the 1920s, more recent knowledge of the mechanisms involved has led to a resurgence of approaches, applied in the perioperative period, to decrease nociceptive input into the CNS. These include more extensive use of pre- and intraoperative analgesics and local anesthetic local and/or regional nerve blocks. A sound understanding of the effects of each addition to the basic approach is needed in order to avoid undesirable drug actions and/or toxicities.

Mechanism of action causing general anesthesia: There have been many attempts to explain the mechanism of general anesthesia at a molecular level. Initially this was aimed at having a unified theory to explain the action of all anesthetics but is now clear that there is a significant difference between most of the injectable and the inhaled anesthetics. Drugs like thiopental, propofol, etomidate, and alfaxalone produce their effects at lower concentrations (nM to μM range) than the inhaled anesthetics that need mM concentrations for their action. It is now known that these injectable anesthetics have their major effect through the $GABA_A$ channel by enhancing the effect of GABA. However, it is also recognized that there are many versions of this receptor, depending on the incorporated subunits, and that specific versions of the receptor may be predominant in some areas of the brain and spinal cord providing a possible basis for species differences in the effects of some of these drugs. The cyclohexanones (ketamine, tiletamine, phencyclidine) are NMDA receptor antagonists and block the actions of the excitatory neurotransmitter glutamate. A unified theory will also have to account for the fact that many of these anesthetics affect the righting reflex, immobility, memory, and nociception, all of which involve different neurotransmitters and receptors. For example, using mice with mutations in the α_1 subunit of $GABA_A$ an effect was demonstrated on the righting reflex while the immobilizing and amnestic effects of isoflurane were unaffected (Sonner et al., 2007). In addition, the diverse chemical structures of inhaled anesthetic agents also pose a problem in arriving at a common theory of action. The inhaled anesthetic drugs range from inert gases such as xenon and relatively simple inorganic (nitrous oxide, carbon dioxide) to organic (isoflurane, sevoflurane) compounds.

For more than a century, two concepts have dominated thinking about the mechanism of action of inhaled anesthetics. Since a wide range of structurally unrelated agents caused anesthesia, Claude Bernard postulated that all of them did so by a common mechanism, the *unitary theory of narcosis* (Leake, 1971). A striking physiochemical characteristic of inhalation anesthetic drugs is their lipid solubility – a physical property shown to correlate best with anesthetic potency. This correlation is commonly referred to as the *Meyer–Overton rule* after the two individuals who independently (in 1899 and 1901, respectively) noted that the potency of anesthetics increased directly in proportion to their partition coefficient between olive oil and water (i.e., the concentration ratio of the agent in oil and water at equilibrium). However, lipid solubility is not the only factor because there are many compounds with lipid solubilities similar to the inhaled anesthetics that do not have any anesthetic affect. Analysis of steric and electrostatic properties of the halogenated inhaled anesthetic drugs shows a structural correlation with potency (Sewell and Sear, 2006) and it is possible that such structural analyses may lead to the development of new anesthetics (Ebalunode et al., 2009). Because inhalation anesthetic molecules are hydrophobic and therefore distribute to sites in which they are removed from aqueous environments, and because of the close correlation between potency and lipophilicity, it was theorized that these anesthetics acted in the cell membrane lipid layer and this idea is still being pursued (Weinrich and Worcester, 2013). The inhalant anesthetics cause immobility through an action in the spinal cord and this is most likely to be in the ventral horn (Kim et al., 2007). In an analysis of the putative receptor mechanisms that could be involved in inhalant immobility, Eger et al. (Eger et al., 2008) eliminate all the commonly suggested receptors except the sodium channel and an, as yet, undiscovered potassium channel. A large part of their argument focuses on the fact that, while many of the inhalant anesthetics have actions at different receptors, the activities are not consistent enough at any single receptor to explain immobility.

Basics of Clinical Anesthesia

A favorable anesthetic course begins with a good plan – a plan based on sound pharmacological and physiological principles. There is no rigid format. *No anesthetic technique is unequivocally the best for all animals under all circumstances.* Each plan is adapted to prevailing circumstances. Accordingly, appropriate anesthetic management requires a broad understanding of the physiology of bodily life support systems (e.g., respiratory, circulatory, central and autonomic nervous systems), the pathology and pathophysiology of the condition(s) necessitating anesthesia and surgery, the pharmacology and principles and techniques of administration of anesthetic and adjuvant drugs, and monitoring and support of vital organ function. The rationale that underlies the selection of an appropriate anesthetic protocol is outlined in Table 9.7.

Drug selection for anesthetic management is accomplished by considering the pharmacological requirements for the individual case, reviewing and selecting major drug classes that are appropriate for the specific needs, and then reviewing characteristics and selecting the specific drug(s) within the desired drug class(es). Frequently, the best drug and technique are those with which

Table 9.7 Considerations in the selection of appropriate anesthetic protocol and drugs

1. Animal characteristics (e.g., species, age, physical status)
2. Capabilities and confidence of anesthetist
3. Capabilities of surgeon and surgical requirements
4. Available drugs, facilities, and ancillary personnel
5. Wishes of client

the clinician is most experienced; that is, there is an art to the clinical administration of potent, life-threatening drugs such as those used in anesthetic management.

Drugs used in anesthetic management can be conveniently classified according to time frame of use: the preanesthetic, perianesthetic, and immediate postanesthetic periods.

Preanesthetic Period

Drugs are usually administered to animals (usually 15–45 minutes) before induction of general anesthesia. The primary aims of preanesthetic medication are to calm the animal, facilitate handling, and relieve preoperative pain. These along with secondary goals are listed in Table 9.8. Unfortunately, preanesthetic medication is not without its complications, which also must be considered in formulating the anesthetic management plan (Table 9.9). The concurrent use of two or three drugs is usually required to accomplish the desired preanesthetic conditions in the patient. These are selected from a variety of major drug classes (Table 9.10). The extent of drug combinations advocated by individuals attests to the variety of circumstances commonly encountered clinically and to the lack of agreement on optimal drug effects.

Tranquilizer-sedatives: Tranquilizers (ataractics or neuroleptics) are frequently administered to animals to produce a calming effect, that is tractability or "chemical restraint." This group of drugs includes the phenothiazine, the butyrophenone, and the benzodiazepine subclasses. They are frequently used in combination with other preanesthetic drugs (e.g., opioids), because lower doses can be used than would be the case if each drug were used alone and the degree of sedation accomplished by the drug combination is often potentiated without causing further severe circulatory and respiratory depression.

Table 9.8 Goals for preanesthetic medication

1. Alleviate or minimize pain
2. Allay apprehension
3. Facilitate handling
4. Minimize undesirable reflex autonomic nervous system activity
    a) Parasympathetic
        i. Vagal nerve
        ii. Secretions: salivary, bronchial
    b) Sympathetic
        i. Arrhythmias
        ii. Arterial blood pressure alterations
5. Supplement general anesthesia
    a) Add to level of analgesia, sedation
    b) Reduce anesthetic requirement
6. Minimize undesirable postanesthetic recovery complications
7. Prevent infection
8. Continue treatment of intercurrent disease

Table 9.9 Complications of preanesthetic medication

1. Depressed vital organ function
    a) Direct effects
    b) Interaction with anesthetic and other adjuvant drugs
2. Antianalgesia
3. Prolonged sedation influencing recovery from anesthesia
    a) Prolonged recumbency
    b) Ataxia

The phenothiazines have been used extensively for decades. They are relatively potent and produce moderately reliable tranquilization. They have antiarrhythmic, antihistaminic, and antiemetic effects that may be

Table 9.10 Major classes of drugs (and specific drug examples) commonly considered for preanesthetic medication

1. Tranquilizer-sedative
    a) Acepromazine
    b) Azaperone
    c) Benzodiazepines
        i. Diazepam
        ii. Midazolam
2. Hypnotic-sedative
    a) Pentobarbital
    b) Chloral hydrate
3. Opioid
    a) Agonist
        i. Morphine
        ii. Meperidine
    b) Agonist-antagonist
        i. Butorphanol
    c) Partial agonist
        i. Buprenorphine
4. α_2-adrenergic agonist
    a) Xylazine
    b) Detomidine
    c) Romifidine
    d) Medetomidine
    e) Dexmedetomidine
5. Dissociative
    a) Ketamine
6. Commercially prepared combinations of sedating drugs
    a) Telazol (tiletamine + zolazepam)
7. Parasympatholytic
    a) Atropine
    b) Glycopyrrolate

particularly desirable. The α_1-adrenergic blocking action of the phenothiazines is likely to be of special concern in some patients because it may result in arterial hypotension.

The butyrophenones also have α_1-adrenergic blocking activity, but appear to be less potent in this regard. Largely on the basis of cost and the lack of broad-based, clear advantages, the butyrophenones are less frequently used for anesthetic management of domestic veterinary patients but are increasingly used in zoo and wildlife settings.

The sedative effects of benzodiazepines, in otherwise healthy animals, are quite variable across animal species commonly encountered in veterinary practice. Consequently, these drugs are commonly used as adjuncts to other preanesthetic drugs in small animals but may be used alone in small ruminants. Pain and occasional erratic absorption after intramuscular injection are characteristic of some benzodiazepines (e.g., diazepam). Sedation caused by benzodiazepines can be reliably reversed by a specific antagonist, at least in some species.

Hypnotic-sedatives: Drugs of this class, including the barbiturates and chloral hydrate, cause a dose-dependent spectrum of CNS depression, sedation, sleep, anesthesia, coma, and death. They produce minimal ventilatory and circulatory depression in sedative doses. Disadvantages of their use include a lack of analgesia and the absence of specific antagonists. Use of drugs from this class have largely been replaced in the preanesthetic period by the α_2-adrenergic agonist drugs.

α_2-Adrenergic drugs: Drugs, such as xylazine, romifidine, and dexmedetomidine, cause dose-related sedation and analgesia. They are widely used across species lines singly and in combination, especially with opioids and dissociative agents. Bradycardia, mild arterial hypertension followed by more prolonged hypotension, hyperglycemia, and increased urine volume are commonly attendant effects. Direct antagonists of varying purity and effectiveness are now available and a peripherally acting antagonist (does not cross the BBB) is being examined with the potential to mitigate most of the cardiovascular effects of these drugs (Honkavaara et al., 2011).

Opioids: Potent analgesia, sedation, and the absence of direct myocardial depression are important advantages of the use of opioids in the preanesthetic period. Patients with preexisting preoperative pain or who will require painful diagnostic or therapeutic procedures before anesthetic induction are likely candidates for opioid preanesthetic medication. Opioid premedication is also appropriate prior to anesthetic management techniques that use opioids as a predominant component (i.e., a balanced technique, see Section General Anesthesia). The main adverse effect of opioids when used prior to anesthesia is the depression of medullary ventilatory control centers resulting in decreased responsiveness to carbon dioxide and in turn hypoventilation. Opioids commonly induce a vagotonic effect so heart rate may also be decreased to variable degrees depending on agent, dose, and animal species. In some species (e.g., the dog) opioids commonly cause CNS sedation, while in others (e.g., the horse) excitement or CNS arousal are predominant concerns. Opioid-induced vomition in some species (e.g., dog) may be wanted (e.g., a newly presented patient with a full stomach requiring anesthesia for a diagnostic or minor surgical procedure) or unwanted (e.g., risk of pulmonary aspiration of vomitus in elderly or depressed patients). They also decrease intestinal propulsive and ruminal activity.

Dissociative drugs: Drugs such as ketamine and tiletamine reliably cause a state of somatic analgesia and sedation in some species (e.g., cat) and may be of benefit in special clinical situations (e.g., highly fractious animals under conditions of limited management choices). Their relatively wide margin of safety in otherwise healthy animals is of special benefit under conditions of limited patient control or understanding of its condition. Ketamine is being used in various combinations in horses and cattle to “stun” the animal to allow brief noxious procedures such as castration or dehorning.

The most prominent disadvantage of their use is that, depending on dose, this class of drugs may cause CNS arousal in some species (e.g., horse) leading to animal excitement or frank convulsions.

Drug combinations: Sometimes, drug combinations are marketed to provide ready access to clinical benefits of two drugs while attempting to minimize their individual disadvantages. For example, Telazol® is a combination of tiletamine, a dissociative agent, and zolazepam, a benzodiazepine tranquilizer-sedative. The combination improves the reliability of the sedative properties of either drug used alone without adding extensively to further vital organ depression (e.g., cardiopulmonary depression). However, as a consequence of the fixed combination, a prolonged duration of effect may be an unwanted result.

Parasympatholytic (anticholinergic) drugs: The most common reason for administering drugs such as atropine or the more potent, longer-acting glycopyrrolate before induction of general anesthesia is to reduce upper-airway and salivary secretions (antisialagogue effect) and counteract reflex bradycardia occurring with, for example, concurrent opioid use or certain surgical manipulations (e.g., ocular).

In years past, it was routine to use anticholinergics as part of the premedication scheme. Although modern inhalation anesthetics tend to decrease airway secretions, it is very common in clinical management of small companion animals to administer opioids as part of anesthetic premedication so anticholinergics may still be considered beneficial in preventing bradycardia. Some argue that heightened vagal tone is best treated just prior to its anticipated occurrence or at first sign of its presence. Sparing use of anticholinergic drugs reduces the risk of other unwanted effects such as tachycardia or reduced gastrointestinal motility. Avoidance of gastrointestinal stasis is of special importance in herbivorous animals, for whom preanesthetic gastrointestinal emptying is almost never desired or accomplished. Mydriasis is another effect (e.g., of atropine) that is often undesirable because it confounds interpretation of some clinical signs of anesthesia and/or exposes the patient to potential retinal damage in some uncontrolled postanesthetic circumstances.

Anesthetic Period

The administration of anesthesia requires a combination of knowledge, skill, and ingenuity. The anesthetic drugs selected and their doses and methods of delivery will largely depend on the animal, the facilities available, and the skill of the individual who will administer them.

General anesthetics are usually given by inhalation or injection; on rare occasions anesthetic drugs may be given orally or per rectum. More specific information on anesthetic delivery is given later in this volume and in cited references (Tranquilli et al., 2006; Dorsch and Dorsch, 2007; Clarke et al., 2014).

Drugs of several classes of injectable agents (Table 9.11) are commonly used for general anesthesia. These are preferably given intravenously (IV); however, because of the varied circumstances associated with clinical conditions in veterinary medicine, the intramuscular (IM) route is also widely used. The IV route is the preferred means of inducing general anesthesia because anesthetic induction with the loss or reduction of many of the patient's life-protecting reflexes is consistently the most crucial maneuver in managing general anesthesia. The IV administration permits incremental dosing and thus titration of the level of anesthetic to a desired end point. This technique is often desired, especially in critically ill patients or in unfamiliar circumstances, because of the likelihood of unpredictable animal responses to a "routine" dose of drug. Drugs of a single class are used alone or in combination with other drugs listed in Table 9.11 (e.g., inhalation anesthetics and neuromuscular blocking drugs) to achieve suitable anesthetic conditions. Many of the drugs from the classes listed in Table 9.11 are also used at lower doses for preanesthetic medication (Table 9.10).

Table 9.11 Major classes of CNS drugs (and examples) commonly considered for general anesthesia

1. Hypnotic-sedatives
    a) Ultrashort-acting (i.e., thiopental)
    b) Short-acting (e.g., pentobarbital)
2. Dissociative
    a) Ketamine
3. Opioid
    a) Morphine
    b) Oxymorphone
    c) Fentanyl
    d) Alfentanil
4. Drug combinations (i.e., Telazol®)
5. Others
    a) Guaifenesin
    b) Propofol
    c) Etomidate
    d) Alfaxalone
6. Tranquilizer-sedatives[a]
    a) Benzodiazepines
        i. Diazepam
        ii. Midazolam

[a] Use as an anesthetic adjuvant in conjunction with others on the list, e.g., opioids or ketamine.

The thiobarbiturates are no longer available in the United States but are still used extensively in other countries. Propofol is used extensively in small animals and the dissociative drugs are used in the most species with almost universal application to anesthetic induction in horses. Alfaxalone has been reintroduced in a new formulation in cyclodextrin and is licensed for use in dogs and cats.

Opioids in large doses are the basis for balanced anesthetic techniques for human patients, especially those patients with circulatory system instability or those undergoing cardiac surgery. This method is also applicable to some veterinary patients (e.g., dogs) and is presently used to varying degrees. An important point to keep in mind is that opioids, even in large doses, do not predictably produce unconsciousness, so other drugs are used concurrently to accomplish the individualized goals of general anesthesia. Also, some animal species (e.g., horses) are excited by even moderate (by comparison to other species, e.g., dogs) opioid doses. The reader should consult Chapter 13 for further discussion.

Immediate Postanesthetic Period

The immediate postanesthetic period is also known as the *anesthetic recovery period*. It begins with the discontinuation of the administration of anesthetic drugs. Recovery of healthy animals from routine anesthetic techniques is usually, but not always, uneventful and routine. Circumstances such as compromised physical status and unfamiliar anesthetic techniques heighten the likelihood of recovery problems. The immediate goal of this

Table 9.12 Hazards of the immediate postanesthetic period

1. Circulatory system complications
   a) Arterial hypotension
   b) Arterial hypertension
   c) Cardiac dysrhythmias
2. Respiratory system complications
   a) Hypoxemia
   b) Hypercapnia (hypoventilation)
3. Pain
4. Emergence excitement (physical trauma)
5. Hyperthermia/hypothermia
6. Vomiting
7. Delayed awakening

period is the rapid return of the patient's independent, uncompromised ability to maintain normal respiratory and circulatory systems function and to return sensory and motor abilities to preanesthetic levels as soon as possible. Despite this overriding philosophy, when the needs of different species and circumstances are considered, the actual broad plan is less clear. For example, most of the contemporary inhalation anesthetics do not have potent or persistent analgesic properties at alveolar concentrations associated with awakening. The sooner a patient recovers following surgery, the sooner there is potential for pain and discomfort for the patient. Consequently, the question arises, is it better for a patient to awaken quickly following surgery and then receive, as needed, analgesic drugs, or is it more desirable and beneficial for the patient to receive analgesic drugs toward the end of the anesthetic period and as a result have a slower recovery from general anesthesia and transition to sensation? The same therapeutic dilemma applies to the patient who may emerge from anesthesia excited and risk a particularly "stormy" recovery with attendant physical injury. The various combinations of drugs used in anesthetic management coupled with unique species characteristics make it impossible to describe here all of the patterns of recovery that occur and appropriate therapeutic schemes. In the end, individualized therapy is the most desirable plan.

Hazards of the recovery period that may require therapeutic intervention are listed in Table 9.12.

Evaluation of the Response to Anesthesia

Since very early in the history of general anesthesia, attempts have been made to correlate observations of the effects of anesthetics with "depth" of anesthesia. To be able to define the depth of anesthesia is important for a number of reasons. For example, too little or too much anesthesia is a threat to life. Consequently, if one can determine the magnitude of anesthesia with reasonable accuracy, patient safety is improved and optimal operating conditions are facilitated for the health care providers. Furthermore, specific guidelines help the novice anesthetist provide appropriate anesthetic conditions. Finally, in investigative circumstances an accurate means for describing and comparing anesthetic levels within or between studies is essential so that we may account for the effects of the anesthetic in our overall understanding versus other variables that may be operant and of interest at the time of study. It would be very helpful to be able to precisely define the depth of anesthesia in every animal from moment to moment regardless of the anesthetic technique. Unfortunately, this is presently not possible, so we rely on estimates.

More than 80 years ago, Guedel (Guedel, 1920, 1927) published his classic description of the four stages of anesthesia. The traditional classification is based on a progressive depression of a continuum of CNS function. Guedel extended the descriptions of earlier workers such as Plomley (Plomley, 1847) and Snow (Snow, 1847) to divide the state of anesthesia into distinct "packages," each correlating with a particular set of physiological responses or reflexes, that is clinical signs. The organizational scheme includes four stages of anesthesia and subdivides the third stage into four strata (i.e., planes) (see earlier editions of this text for a broader description). Guedel's system has been prominent in pharmacology texts (including earlier editions of this text) and anesthesia texts for more than six decades. The concept is included here in only abbreviated form because with newer drugs and changes in clinical practice its importance in the discussion of fundamental principles of anesthesia is limited.

The classic signs and stages are partly recognizable with many general anesthetics (e.g., the barbiturates), but they are incomplete or are obscured when using modern anesthetics (e.g., ketamine) and/or techniques. It is important to remember that Guedel's description was based on his observation of the actions of diethyl ether administered to otherwise unmedicated human patients who were breathing spontaneously. This is a situation far different from contemporary clinical practice (including veterinary), in which controlled mechanical ventilation is common and newer and multiple anesthetic and adjuvant drugs are an important part of the anesthetic plan. There are unique differences in the way different species react to conditions of general anesthesia that also must be taken into consideration. Because of diethyl ether's characteristics and the methods of delivery, the onset and "deepening" of anesthesia were slow. This situation facilitated a slow (relative to today's standards) unfolding. In addition, numerous physiological responses to anesthetics that are widely monitored today (Table 9.13) are not included in the classic description.

With the emergence of anesthetics such as ketamine and enflurane (absent from veterinary practice for more

Table 9.13 Useful signs in clinical assessment of anesthetic depth. Source: Steffey 1983. Reproduced with permission of Springer.

1. Cardiovascular system
   a) Heart rate and rhythm[a]
   b) Arterial blood pressure[b]
   c) Mucous membrane color
   d) Capillary refill time
2. Respiratory system
   a) Breathing frequency[a]
   b) Ventilatory volumes (tidal and minute ventilation)[a]
   c) Character of breathing[a]
   d) Arterial or end-tidal CO_2 partial pressure[b]
3. Eye
   a) Position and/or movement of eyeball[b]
   b) Pupil size[a]
   c) Pupil response to light
   d) Palpebral reflex
   e) Corneal reflex
   f) Lacrimation
4. Muscle
   a) Jaw or limb tone[a]
   b) Presence or absence of gross movement[a]
   c) Shivering or trembling[a]
5. Miscellaneous
   a) Body temperature
   b) Laryngeal reflex[a]
   c) Swallowing[a]
   d) Coughing[a]
   e) Vocalizing[a]
   f) Salivating
   g) Sweating
   h) Urine flow

[a]Moderate or [b]high specificity in assessment of anesthetic depth for various animal species and anesthetic agents.

than two decades), the concept that all anesthetics are depressants required reconsideration. Winters et al. (1967) proposed replacement of the classic, unidirectional schema (Figure 9.9) of CNS excitation and depression with a new schema that included a description of progressive states of both CNS depression and excitation (Figure 9.10). The new schema recognized bidirectional influences of drugs acting on the CNS and was based on results of electrophysiological studies of anesthetic, excitatory, hallucinogenic, and convulsive agents in cats (Winters et al., 1967; Winters et al., 1972; Winters, 1976).

Guedel's scheme also does not take into consideration the modifying influences of such things as duration of anesthesia (Dunlop et al., 1987; Steffey et al., 1987a, 1987b) or varying magnitudes of surgical stimulus intensity on the signs of anesthesia (Eger et al., 1972; Steffey, 1983). In modern clinical anesthetic practice it is recognized that no single observation is always reliable as a sign indicating a specific magnitude of anesthesia. Accordingly, anesthetists are encouraged to develop a basic background knowledge of both the individual to be anesthetized and the selected drugs. Current advice for anesthetic management under clinical conditions is to use an initial anesthetic loading dose just necessary

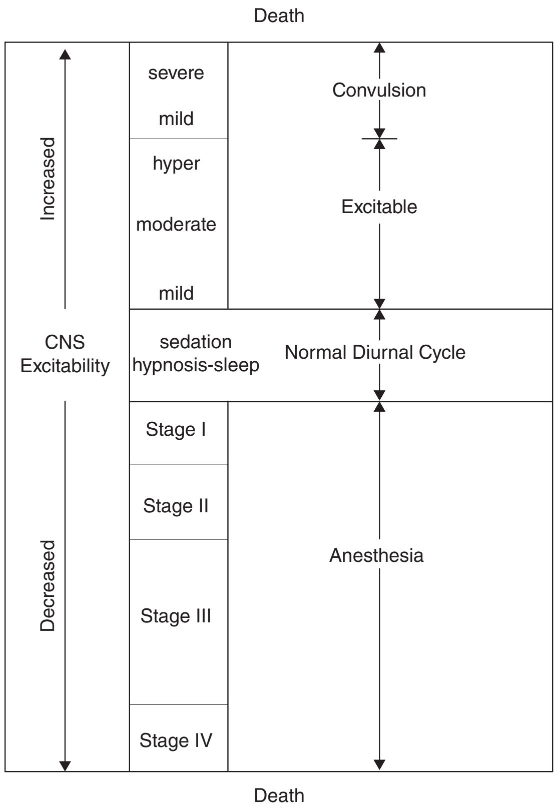

Figure 9.9 The classic unidimensional schema of CNS excitation and depression. Source: Adapted from Winters, 1976.

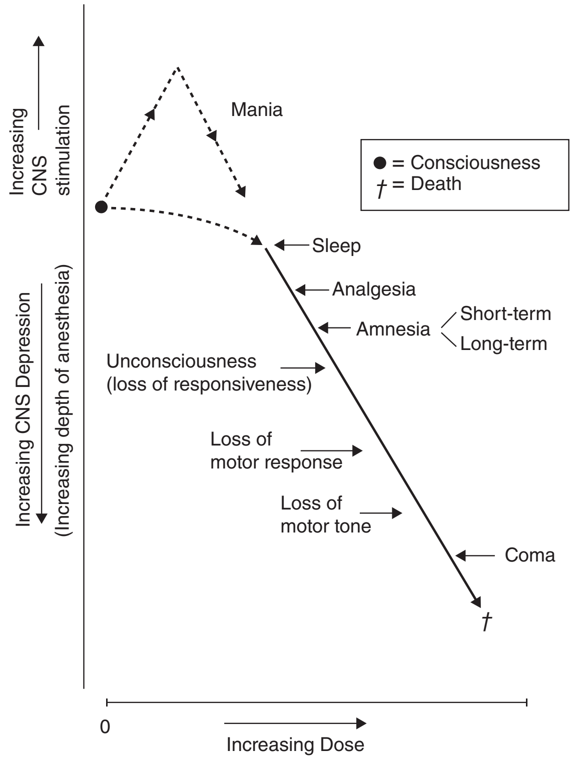

Figure 9.10 The anesthetic continuum as viewed clinically in a healthy subject.

to suppress purposeful movement and to observe all signs possible in each patient (Table 9.13) and then to manipulate further anesthetic dose in relation to continual stimulus–patient response assessment. If the level of anesthesia is in doubt, err on the side of an animal that is too lightly anesthetized.

Stimulus assessment is of special importance since the intensity of stimulus applied to an anesthetized animal may rapidly and markedly alter the observed signs (Eger et al., 1972; Steffey, 1983; March and Muir, 2003). A quiet animal with reasonable vital signs may quickly show evidence of light to moderate anesthesia in the presence of intense visceral stimulation despite no change in anesthetic delivery. Common responses to anesthetic dose–stimulus interaction are given in Table 9.14.

More precise quantitative measures of anesthetic depth are of obvious interest for the clinician and essential in research. Measurement of end-expired (alveolar) concentration of inhaled anesthetics allows the clinician to estimate anesthetic depth based on knowledge of the patient and concurrent drug administration but clinical signs are still important. There is continued interest in using electrophysiological approaches to measure depth of anesthesia in both the laboratory and the operating room. For example, it is long known that anesthetic drugs alter the spontaneous electroencephalogram and other electrophysiological measures of CNS function. Notable use of this information is the development of bispectral EEG signal processing for monitoring anesthetic depth (Rampil, 1998). The bispectral index (BIS) is a complex, proprietary EEG parameter that was approved by the US Food and Drug Administration for commercial availability as a monitor of anesthetic effect in human patients. Studies using such technology have shown good correlations with clinical signs in a number of animal species (March and Muir, 2003) but its general application will have to await more detailed information and a decrease in the cost of the equipment. For a more complete review of central nervous system–focused electrophysiological technology applied to anesthetic management of human patients, see Stanski and Shafer 2005.

Table 9.14 Common responses to anesthetic dose stimulus interaction. Source:Steffey 1983. Reproduced with permission of Springer.

1. Signs of presurgical anesthesia
   a) Bradycardia, tachycardia, arrhythmia
   b) Arterial hypertension
   c) Pupillary dilation, lacrimation, globe rotation
   d) Tachypnea or breath holding
   e) Deep breathing
   f) Reduced alveolar/arterial $P\text{CO}_2$
   g) Limb/body movement
   h) Salivation, vomition
   i) Swallowing
   j) Laryngeal spasm
   k) Phonation
2. Signs of deep surgical anesthesia
   a) Bradycardia, tachycardia, arrhythmia, cardiac arrest
   b) Arterial hypotension
   c) Pupillary dilation, dry cornea, centrally fixed eye
   d) Shallow breathing, respiratory arrest (*not* breath holding)
   e) Elevated alveolar/arterial $P\text{CO}_2$
   f) Muscle flaccidity

Note: Importance of a given sign in a specific species and/or individual varies.

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10

Neuromuscular Blocking Agents

Diane E. Mason and Christopher L. Norkus

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Neuromuscular blocking agents used in clinical medicine act by interfering with the action of the endogenous neurotransmitter acetylcholine (ACh) on the nicotinic cholinergic receptor at the neuromuscular junction (NMJ), thereby inhibiting receptor-coupled transmembrane ion movements necessary to initiate muscle contraction (Bouzat et al., 2004; Unwin, 2005). The end result of this action is skeletal muscle paralysis and muscular relaxation (Bowman, 2006). Neuromuscular blocking (NMB) agents are used in veterinary medicine as adjuncts to general anesthesia. The most common indications for a NMB agent in veterinary medicine are to aid in the placement of an endotracheal tube, to be used concurrently with sedative/hypnotic agents to facilitate mechanical ventilation, to enhance muscle relaxation for a variety of surgical conditions (e.g. ophthalmological procedures), or as part of a balanced anesthetic technique to reduce the amount of inhalation anesthetic required (Keegan, 2015). The sporadic use of NMB agents in veterinary medicine is in stark contrast to the widespread use of NMB agents in human anesthesia and is largely a result of interspecies differences. For example, most species seen in veterinary medicine can be easily intubated with an endotracheal tube after administration of an intravenous anesthetic induction agent without the additional need of a NMB agent, while intubation in man is often more difficult and may necessitate deeper muscle relaxation (Bozeman et al., 2006).

Development

Development of NMB drugs represents a colorful history in the field of pharmacology. Interesting reviews of the course of these events have been presented by Betcher (1977), Bisset (1992), and Lee (2005). Neuromuscular blocking drugs originated with the discovery of curare, a tarlike mixture of plant material used as a poison by South American Indians. The actual ingredients of the poison for arrows, blowgun darts, and spears were known only to a local "pharmacist," who was often the tribal medicine man of a region. Thus the botanical preparations obtained by explorers could not be identified as to content; they were simply classified according to the containers in which they were packaged. Tubo-, para-, or bamboo-curare was contained in cutoff bamboo tubes; this mixture was usually obtained from southern Amazon tribes. The plant origin of tube-curare preparations was primarily Menispermaceae (*Chondrodendron tomentosum*). Calabash-curare was packaged in hollow gourds or calabashes; it was the most active preparation. Pot-curare came in small earthenware pottery from the central part of the Amazon basin; this concoction often contained plants other than Menispermaceae (McIntyre, 1972). The most important constituent isolated from curare was *d*-tubocurarine (Wintersteiner and Dutcher, 1943).

Original studies in the 19th century by Claude Bernard (1856) (Bowman, 2006) demonstrated that curare prevented the muscle contraction elicited by stimulation of the motor nerve. It did not, however, affect the central nervous system (CNS), prevent response to direct stimulation of the muscle, or depress axonal conductance. It was proposed that curare acted at the nerve–muscle junction. Reports since then have substantiated, clarified, and extended observations concerning the neuromuscular blocking properties of curare alkaloids. Early results stimulated active research into the chemical structural requirements of curare-like compounds, leading to the discovery of other types of NMB agents.

Neuromuscular blocking agents possess chemical structural groups that allow interaction of these agents with the nicotinic cholinergic receptor (Brejc et al., 2001). However, these drugs cause distinctly different effects from the endogenous mediator ACh. According

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to the drug's mechanism of action at the nicotinic postjunctional receptor, NMB drugs are classified as either competitive nondepolarizing agents or as depolarizing agents. Competitive nondepolarizing NMB agents occupy the receptor so that ACh cannot access its binding site and upon binding these compounds fail to trigger transmembrane ion movement, resulting in muscle paralysis for the duration of their effect. Depolarizing agents act in a more complicated manner and initially cause membrane depolarization, often characterized by muscle fasciculation, before blockade and muscle paralysis occur (Hibbs and Zambon, 2011).

Impulse Transmission at the Somatic Neuromuscular Junction

Prior to discussing individual NMB agents, impulse transmission at the somatic NMJ will be reviewed in relation to sites of action of different drugs. General concepts of cholinergic transmission are discussed in detail in Chapters 6 and 8.

Physiological and Anatomic Considerations

A representation of a somatic NMJ synapse and proposed sites of drug actions are shown in Figure 10.1. Terminal branches of a motor axon lose their myelin sheath and embed within invaginations of the cell membrane of the skeletal muscle cell; these invaginations are termed synaptic gutters. A synaptic gutter, in turn, has many microinvaginations or infoldings, called junctional folds. The space within the synaptic gutter between the nerve ending and the muscle cell is called the synaptic cleft. *Presynaptic* refers to nerve axon elements, whereas *postsynaptic* refers to constituents of the muscle cell.

Vesicular structures localized within cholinergic nerve terminals represent storage sites for ACh (see Chapter 6). As an axonal action potential arrives at the nerve terminal, it increases the release of ACh from the storage vesicles into the synaptic cleft. This step (excitation–secretion coupling) is dependent upon an action potential activating voltage-gated Ca^{++} channels that shift extracellular Ca^{++} into the neuron and/or release Ca^{++} bound to superficial membranes of the nerve terminal. The rise in intracellular Ca^{++} triggers fusion of the storage vesicles with the plasma membrane, and neurotransmitter release. The ACh released into the synaptic cleft binds to specialized receptor sites on the postsynaptic membrane and causes depolarization of the muscle cell. The exclusively nicotinic cholinergic receptors located on the plasma membrane surface of the muscle cell are clustered in high density in the

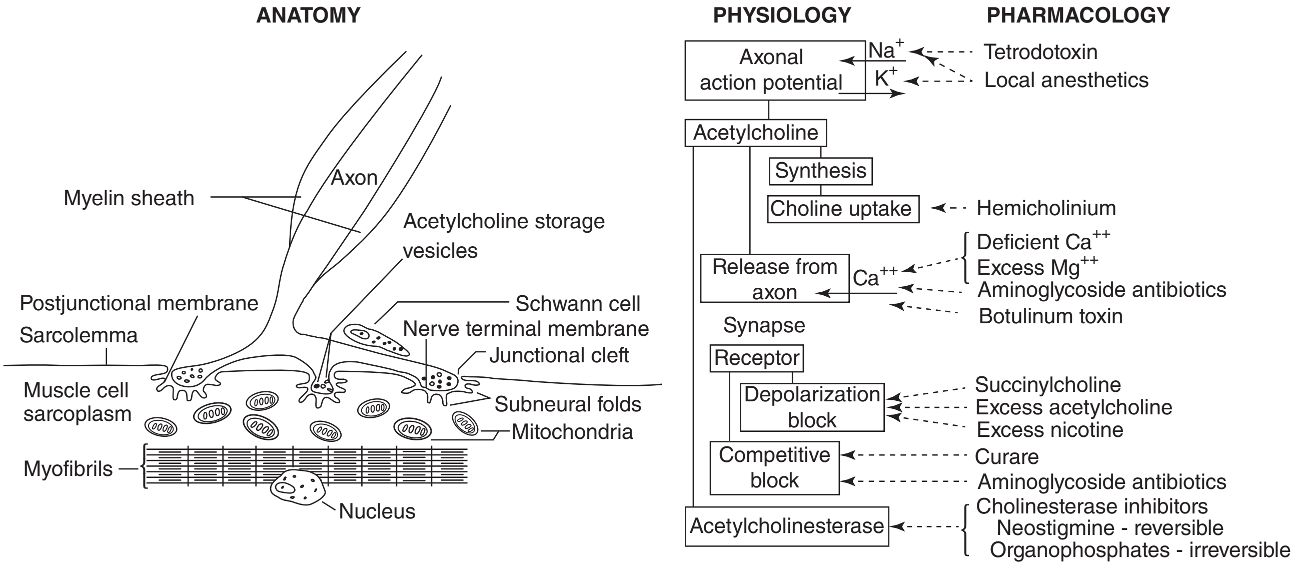

Figure 10.1 Schematic representation of a somatic neuromuscular junction (synapse), related physiological pathways, and proposed sites of action of various pharmacological agents. An axonal action potential (AP) is characterized by an influx of Na^+ and an efflux of K^+. Tetrodotoxin and saxitoxin inactivate Na^+ pathways. Local anesthetics block Na^+ and K^+ pathways. Choline uptake into the neuron is blocked by hemicholinium; synthesis of ACh is prevented. As the AP arrives at the nerve terminal, it instigates inward movement of Ca^{++}; this triggers discharge of ACh into the junctional cleft. A lack of Ca^{++} or an excess of Mg^{++} decreases release of ACh. Aminoglycoside antibiotics also interfere with Ca^{++} -dependent release of ACh. Botulinum toxin inhibits ACh release. Succinylcholine (depolarizing neuromuscular blocking agents) cause persistent depolarization block of the motor end-plate region, as does excess ACh and nicotine. Curare and atracurium (competitive neuromuscular blocking agents) compete with ACh for postsynaptic receptors but do not cause depolarization. Aminoglycoside antibiotics decrease sensitivity of the postsynaptic membrane to ACh. Catabolism of ACh by acetylcholinesterase is inhibited by reversible and irreversible anticholinesterase agents; ACh accumulates. Source: Modified from Hibbs and Zambon, 2011; Couteaux, 1972.

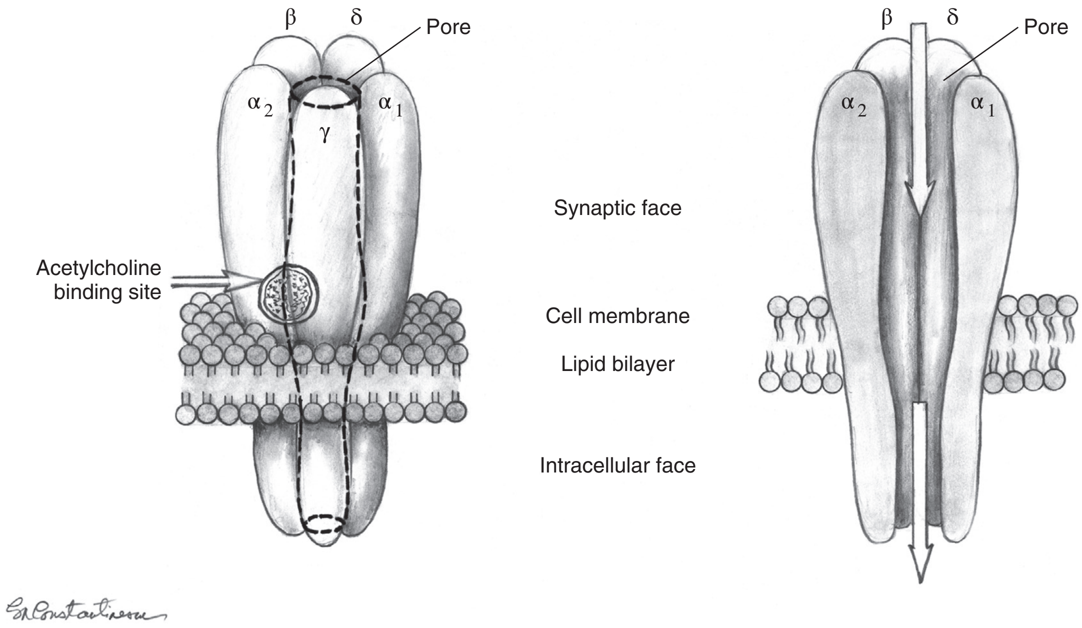

Figure 10.2 Schematic representation of the nicotinic cholinergic receptor. The receptor is embedded across the cell membrane lipid bilayer, presenting a synaptic face to the neuroeffector junction between the neuron and innervated cell, and an intracellular face within the cytoplasm. The receptor comprises a pentameric configuration of four separate subunits with a stoichiometric ratio of $\alpha_2\beta\gamma\delta$; in adult muscle $\alpha_2\beta\varepsilon\delta$. The α subunits contain the primary ligand binding sites for recognition of acetylcholine and related agents. Subunit arrangement forms an internal pore that allows passage of select ions upon receptor activation and resulting membrane depolarization (see text). Source: Redrawn from Unwin et al. (1988) by Dr. Gheorghe M. Constantinescu, University of Missouri.

junctional folds of the postsynaptic membrane (Huh and Fuhrer, 2002).

It requires the activation of a large number of nicotinic cholinergic receptors to excite a single muscle fiber and stimulate muscle contraction. At the same time the process requires a very rapid termination of response. A single synaptic vesicle contains roughly 7000–12,000 molecules of ACh and a single motor axon action potential may trigger the fusion of 40–300 vesicles depending on the species or type of NMJ studied (Steinbach and Wu, 2004). After release into the synaptic cleft the ACh reaches high concentration rapidly, where it can bind to cholinergic receptors. However, ACh released into the synaptic cleft also can bind to the enzyme acetylcholinesterase (AChE), which hydrolyzes ACh to choline and acetate thus inactivating it. All unbound, extraneuronal ACh is rapidly metabolized by the AChE enzyme, which is localized in the motor end-plate region. Although AChE may be bound in part to presynaptic elements, it is concentrated at the postsynaptic membrane (Inestrosa and Perelman, 1990; Hucho et al., 1991). The relative ratio of binding sites for ACh on the nicotinic cholinergic receptor to AChE binding sites at the NMJ is approximately 10 : 1 (Steinbach and Wu, 2004). Due to the rapid rate of hydrolysis of ACh by AChE and the comparatively slow release of ACh from relatively large numbers of nicotinic receptors at the motor end-plate, the concentration of free ACh within the synaptic cleft is rapidly reduced after a single action potential.

The Nicotinic Receptor and Structure–Activity Relationships

The nicotinic cholinergic receptor is a pentameric molecule of about 290 kilodaltons that spans the bilayer of the postsynaptic membrane at the NMJ (Figure 10.2). The receptor comprises five individual subunits in a stoichiometric ratio of α2βγδ; the γ-subunit is replaced by an ε-subunit in muscle from adult animals. Each subunit presents an extracellular and intracellular surface and also contains sequences of hydrophobic amino acids that are the likely regions embedded within the membrane bilayer (Hibbs and Zambon, 2011). The five subunits of each individual receptor complex are elongated perpendicular to the postsynaptic membrane and are arranged circumferentially to form a rosette around a central lumen (Figure 10.2). This central transmembrane channel of the receptor complex represents the membrane pore for ion fluxes instigated by agonist activation of the receptor (Unwin, 2005). Agonist and antagonist binding sites are restricted to the α-subunits (Kistler et al., 1982). Whereas ACh evokes receptor activation upon binding to the α-subunits, occupation of these same sites by antagonists prevents effective receptor activation

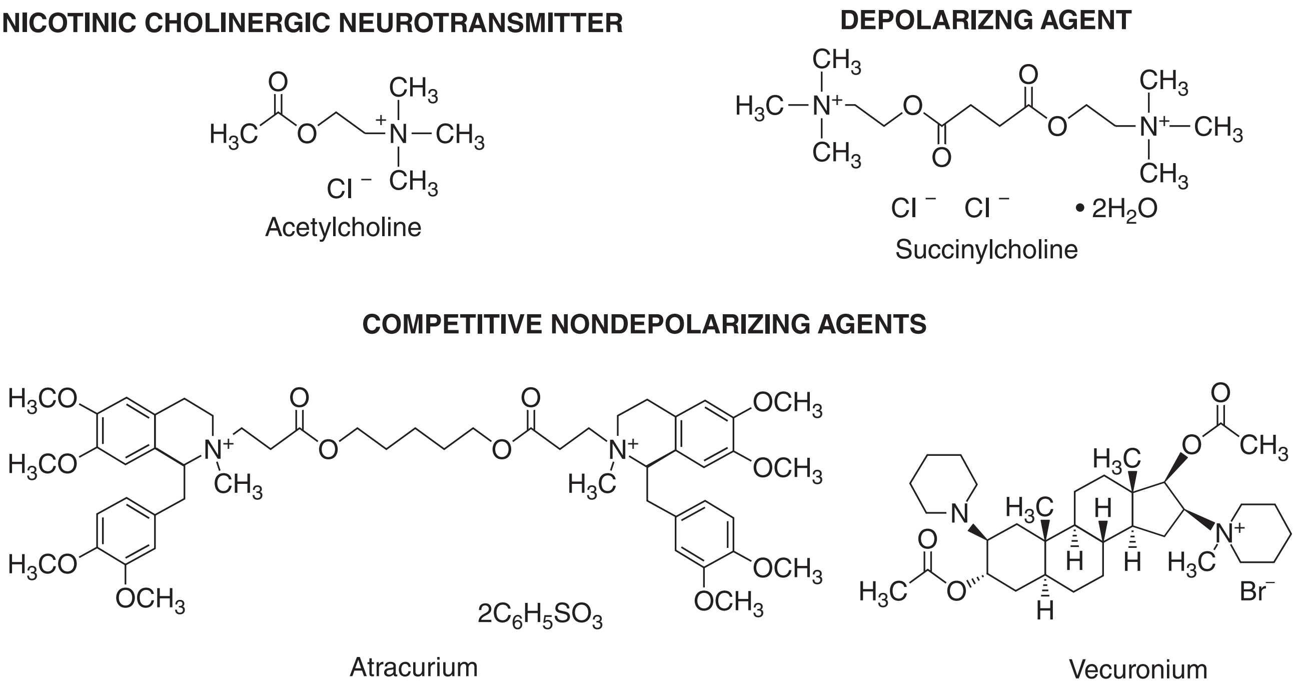

Figure 10.3 Chemical structures of agents interacting with the nicotinic cholinergic receptor at the neuromuscular junction. The cholinergic neurotransmitter, acetylcholine, contains a cationic nitrogen moiety that forms a bond with receptor binding site. Two molecules of acetylcholine must bind to a nicotinic receptor to activate the ion channel. The depolarizing neuromuscular blocking agent, succinylcholine, is depicted. The chemical structure of succinylcholine is equivalent to two acetylcholine molecules fused in sequence. A single molecule of succinylcholine can bind to both of the nicotinic binding sites on the cholinergic receptor inactivating the receptor. Competitive nondepolarizing NMB agents from the benzylisoquinolinium family (atracurium) and the aminosteroid group (vecuronium) are represented. These compounds are complex bulky molecules but contain the important dual cationic nitrogen moieties in a rigid configuration that allows occupation of both of the nicotinic binding sites on the cholinergic receptor and effectively inactivating the receptor.

(Karlin, 2002). The muscle becomes paralyzed, whether in response to a competitive nondepolarizing blocking agent or to transient activation by a depolarizing blocking agent. Chemical structures of several commonly used NMB agents are shown in Figure 10.3 to demonstrate structural differences of the competitive, nondepolarizing and the depolarizing types of NMB agents.

Based on general chemical structural characteristics, Bovet (1951) placed neuromuscular blocking agents into two basic categories. One group is characterized by large, bulky, rigid molecules; members of this group include *d*-tubocurarine, vecuronium, atracurium, and pancuronium, all of which are the competitive, nondepolarizing neuromuscular blockers. The other group is characterized by long, slender, flexible molecules that allow free bond rotation. Decamethonium and succinylcholine are in this group; these agents represent member of the depolarizing NMB agents. The dichotomy in basic structural arrangement of competitive, nondepolarizing and depolarizing agents has been offered as a partial explanation for dissimilar effects evoked by interaction of these agents with the nicotinic cholinergic receptor.

Among other requirements, receptors contain two anionic (negatively charged) binding sites separated by set distances. These sites are essential for electrostatic bonding of the cationic (positively charged) nitrogen moiety of ACh (and exogenous chemicals) to the receptors (Brejc et al., 2001). All NMB agents, are quaternary ammonium compounds, containing the cationic nitrogen regions necessary to interact at the ACh binding site. Despite the variety of chemical structures represented among the modern nondepolarizing NMB agents, a maximum bond distance between the two quaternary groups present in any of these molecules is typically fixed at 1.0 ± 0.1 nm. Depolarizing NMB agents that vary in length and have free bond rotation may have a distance between quaternary ammonium groups up to a maximum bond distance of 1.45 nm (Hibbs and Zambon, 2011).

Occupation of negatively charged binding sites on the receptor by the neurotransmitter, ACh activates influx of Na^+ and flux of K^+ along their respective concentration gradients, resulting in membrane excitation. Occupation of these sites by the molecularly rigid competitive agents stabilizes the receptor so that the membrane channel is not activated. Depolarizing agents initially act similarly to ACh. Because of their flexible structure, they allow initial channel activation and ion flow but for some reason result in a persistent interruption in ion flow through the receptor so that additional changes in electrical potential are not achieved.

Pharmacological Considerations

The NMJ is quite susceptible to alteration by selective pharmacological agents. Various drugs, toxins, electrolytes, and other agents alter in different manners the synthesis, storage, release, receptor interactions, and catabolism of ACh. Several important factors affecting cholinergic transmission are outlined in Figure 10.1.

Hemicholinium is a choline-transport inhibitor that interferes with choline reuptake into cholinergic neurons; although this agent has no current clinical application, it is used widely in research application (Inazu et al., 2013). Acetylcholine synthesis is prevented by lack of choline, classified as an essential nutrient in the B vitamin complex family (Ferguson and Blakely, 2004). Existing vesicular stores of ACh are exhausted upon nerve stimulation, and a gradual weakening and eventual paralysis will result without reuptake and synthesis.

Nerve conduction is affected by only a few substances. Local anesthetics in immediate contact with the motor nerve axon act to stabilize the nerve by inactivating both Na^+ and K^+ channels so that axonal action potential propagation is halted. The puffer fish poison tetrodotoxin and the shellfish poison saxitoxin decrease the permeability of excitable membranes to Na^+ (but not K^+); thus axonal action potentials are not generated and paralysis results. These toxins do not cause an initial depolarization of nerves; they act noncompetitively, are approximately 100,000 times more potent than cocaine or procaine, and are frequently used in research. Clinical cases of fatal food poisoning have also been attributed to ingestion of these substances from their natural source (Cusick and Sayler, 2013).

Botulinum toxin is a potent substance (lethal dose for a mouse is 4×10^7 molecules) produced by *Clostridium botulinum.* It is ingested rarely by humans, more often in cattle, horses, poultry and waterfowl and often is fatal (Johnson et al., 2010). It acts within the motor axon to prevent ACh release into the NMJ by interfering with fusion of cholinergic synaptic vesicles with the plasma membrane (Dressler et al., 2005).

Magnesium ions (Mg^{++}) interfere with release of ACh from the nerve terminal by competing for the transport mechanisms responsible for mobilization of Ca^{++} into the nerve. Magnesium uncouples the excitation–secretion coupling process. An insufficient concentration of Ca^{++} produces similar effects. Magnesium also acts postsynaptically to decrease the effectiveness of ACh to activate receptors.

Aminoglycoside antibiotics (i.e., neomycin–streptomycin group) inhibit release of ACh from motor nerves by decreasing availability of Ca^{++} at superficial membrane binding sites of the axonal terminal, thereby inhibiting the excitation–secretion coupling process. These antibiotics also reduce sensitivity of the postsynaptic membrane to ACh (Adams, 1984).

Cholinesterase inhibitors (see Chapter 8) decrease the hydrolytic activity of AChE and pseudocholinesterase (Taylor, 1991; Hibbs and Zambon, 2011). ACh rapidly accumulates at receptor sites. Muscle fasciculations, spasms, convulsions, and eventually apnea occur after overdosage with cholinesterase inhibitors.

Postjunctional Mechanisms of Neuromuscular Blockade

The pharmacological effects of clinically useful NMB drugs are a result of their direct alteration of the ability of ACh to activate postsynaptic nicotinic cholinergic receptors (Hibbs and Zambon, 2011).

Competitive Nondepolarizing Agents

Nondepolarizing NMB agents compete with ACh for available nicotinic cholinergic receptors at the neuromuscular postsynaptic membrane and, once occupying these receptors, prevent the ability of Ach to produce a motor response. The historical prototype of this group of drugs is *d*-tubocurarine (tubocurarine chloride, USP, Tubarine), a naturally occurring monoquaternary alkaloid obtained from the bark of the South American plant *Chondrodendron tomentosum*. Today *d*-tubocurarine is not used clinically and has long been replaced with similarly acting agents, including atracurium, cisatracurium, pancuronium, vecuronium, rocuronium, mivacurium, doxacurium, and, most recently, gantacurium. Pharmacological characteristics of representative NMB agents in modern use are summarized in Table 10.1.

Ultra refined experimental techniques helped verify the primary site of action of competitive blocking agents (Bowen, 1972; Hubbard and Quastel, 1973). Although *d*-tubocurarine binds to cholinergic receptors in the same region as ACh, and appears to have the same or similar affinity as ACh for cholinergic receptors, *d*-tubocurarine does not exhibit receptor-activating properties, it has no depolarizing activity, and therefore does not cause a motor end-plate potential. Moreover, the *d*-tubocurarine–receptor interaction renders affected receptors unavailable for interaction with ACh. ACh-induced end-plate potentials are reduced to subthreshold levels or abolished in curarized muscles. In the absence of induced end-plate potentials and subsequent muscle action potentials, the muscle relaxes and is, in fact, paralyzed.

Based on competitive interaction between nondepolarizing agents and ACh, cholinesterase inhibitors were

Table 10.1 Characteristics of neuromuscular blocking agents

Drug	Onset	Duration	Elimination	Dosing	Constant rate infusion
Depolarizing agents					
Succinylcholine	1–2 min	6–10 min	Hydrolysis by plasma cholinesterase	0.3–0.4 mg/kg (C) 0.1–0.2 mg/kg (F) 0.12–0.15 mg/kg (E)	
Competitive nondepolarizing agents					
Atracurium	5 min (C)	30–40 min (C)	Ester hydrolysis, Hofmann elimination	0.1–0.2 mg/kg (C) 0.1–0.25 mg/kg (F) 0.07–0.15 mg/kg (E)	0.18–0.36 mg/kg/h (C) 0.17 mg/kg/h (E)
Cisatracurium	5 min (C)	30–40 min (C)	Hofmann elimination	0.075–0.3 mg/kg (C) 0.05–0.3 mg/kg (F)	
Doxacurium	40 min (C)	100–120 min (C)	Renal and biliary elimination of unchanged drug	3.5 μg/kg (C)	
Vecuronium	5 min (C)	30–40 min (C)	Hepatic metabolism biliary and renal excretion	0.1 mg/kg (C) 0.025–0.1 mg/kg (F) 0.1 mg/kg (E)	
Rocuronium	1–2 min (C)	20–30 min (C)	Hepatic uptake and Biliary excretion	0.1–0.6 mg/kg (C) 0.1–0.6 mg/kg (F) 0.3–0.6 mg/kg (E)	0.2 mg/kg/h (C)
Pancuronium	5 min (C)	30–60 min (C)	Hepatic metabolism and renal elimination of active metabolites	0.07–0.1 mg/kg (C) 0.06–0.1 mg/kg (F) 0.12 mg/kg (E)	
Gantacurium	1–2 min (C)	5 min (C)	Ester hydrolysis, cysteine adduction	0.06 mg/kg (C) 0.06 mg/kg (F)	

Species indicated are canine (C), feline (F) and equine (E).

found to be effective in antagonizing the effects of these blocking agents (Taylor, 2011). Cholinesterase inhibitors prevent the enzymatic catabolism of ACh. More ACh is available for interaction with cholinergic receptors and thereby decreases effectiveness of competitive blocking agents. This relationship has been exploited clinically in successful efforts to terminate the effects of nondepolarizing agents. However, cholinesterase inhibitors do not antagonize effects of the other class of neuromuscular blockers, the depolarizing drugs.

Nondepolarizing NMB agents are classified as pachycurares, or bulky molecules having their amine functions incorporated into rigid ring structures. Two groups of synthetic pachycurares contain the drugs in common use in medicine today: the aminosteroids and the benzylisoquinoliniums. The aminosteroids maintain their interonium distance by an androstane skeleton while the benzylisoquinoliniums maintain this atomic distance by linear diester-containing chains (Lee, 2001).

Benzylisoquinolinium Compounds

Atracurium is a *bis*-benzyltetrahydroisoquinolinium with isoquinolinium nitrogens connected by a diester-containing hydrocarbon chain. Its action is intermediate with a dose-dependent onset of action of approximately 5 minutes and duration of approximately 30 minutes in dogs (Jones, 1983). Repeated doses are generally not cumulative, so longer-term maintenance can be achieved via a constant-rate infusion (Playfor et al., 2000). Atracurium undergoes ester hydrolysis, Hofmann elimination reaction, and likely other nonhepatic routes for biotransformation, making the drug appropriate for use in hepatic or patients with renal insufficiency. In a Hofmann elimination reaction, a quaternary ammonium group is converted to a tertiary amine by cleavage of a carbon–nitrogen bond. This process does not require enzymatic activity. Hofmann elimination is both a pH and temperature-sensitive reaction in which higher pH and temperatures favor drug breakdown. Clinical consequences of this are important in that patient acidemia and hypothermia may hinder drug breakdown (Playfor et al., 2000). Additionally, the drug should be kept refrigerated and is supplied at a pH of 3.25–3.65 to slow degradation.

The marketed atracurium product has ten isomers. These isomers are separated into three geometric isomer groups that have been designated cis–cis, cis–trans, and trans–trans according to their configuration about the tetrahydroisoquinoline ring system (Wastila et al., 1996). Cisatracurium is the 1R cis-1'R cis isomer of atracurium and represents approximately 15% of the marketed atracurium product's weight yet produces roughly 50% of atracurium's neuromuscular blocking activity. Cisatracurium (Nimbex) is available as a standalone product separate from atracurium. Cisatracurium is metabolized by Hofmann elimination entirely. The presence of portosystemic shunt and hepatic insufficiency did not affect the rate of onset or duration of action of cisatracurium in dogs receiving 0.1 mg/kg IV followed by repeat doses of 0.03 mg/kg IV (Adams et al., 2006). In general, cisatracurium has a similar duration and clinical effects to atracurium.

Following intravenous injection, atracurium spontaneously decomposes into laudanosine and a quaternary monoacrylate. Laudanosine in high quantities is a CNS stimulant and may result in seizures, hypotension, and tachycardia (Chapple et al., 1987). Laudanosine, unlike atracurium, is dependent upon hepatic clearance, so theoretically laudanosine plasma concentrations may become elevated in patients with hepatic dysfunction. Practically, laudanosine-induced toxicity is unlikely in clinical patients unless atracurium is used in large doses and/or for prolonged periods (Chapple et al., 1987). Nonetheless, another aminosteroid may be selected to avoid this remote issue entirely.

d-Tubocurarine, the prototypical benzoisoquinolone NMB agent, is associated with histamine release and resulting hypotension. Although atracurium has the potential to result in histamine release, this requires several times the ED_{95} dose (effective dose producing a 95% reduction in twitch height) before appreciable amounts of histamine are released, making problems such as hypotension and tachycardia rarely observed in clinical cases (Scott et al., 1986; Hackett et al., 1989). In isoflurane-anesthetized dogs, 0.2 mg/kg IV atracurium did not result in significant changes to intraocular pressure, mean arterial pressure, heart rate, or central venous pressure (McMurphy et al., 2004). A notable exception to atracurium and cisatracurium's minimal cardiovascular profile may be in dogs with X-linked muscular dystrophy. Affected golden retrievers in one retrospective study that received 0.1 mg/kg cisatracurium had marked increases in heart rate (115 ± 64%) and blood pressure (33.5 ± 31%) that lasted 10 and 30 minutes, respectively (Staffieri et al., 2011).

Atracurium has been widely used with positive clinical success across a variety of species including, but not limited to, dogs, cats, and horses. In horses and dogs, the use of atracurium in conjunction with aminoglycoside antibiotics, specifically gentamycin, has been shown to augment neuromuscular blockade; however, this effect appears minimal and has not been reported to impact recovery quality (Hildebrand and Hill, 1994; Martinez et al., 1996). Atracurium has also been investigated for use in a urethral flushing solution in cats and dogs to help facilitate manual bladder expression in patients with spinal cord injuries and in obstructive urethral plugs (Galluzzi et al., 2012; Galluzzi et al., 2015).

Two additional benzylisoquinolinium compounds, mivacurium and doxacurium, are reported in the veterinary literature but are infrequently used in clinical practice today. Mivacurium (Mivacron) is a short-acting NMB agent in humans (15–20 minutes' duration); however, this duration of effect appears markedly prolonged in dogs. Although mivacurium resulted in minimal hemodynamic changes, in dogs administered 0.05 mg/kg IV it appeared to have a long half-life and slow clearance under halothane anesthesia with duration of effect up to 151.0 ± 38.50 minutes (Smith et al., 1999a, 1999b). Clinical observations in cats suggest that mivacurium has a shorter duration of action compared to that in dogs. Mivacurium is unique amongst the benzylisoquinolinium compounds in that it is metabolized by plasma cholinesterases to a monoester and this attribute, as a result of interspecies differences in plasma cholinesterases, may explain its variable duration of action between species. At the time of writing, mivacurium is not currently available within the United States.

Doxacurium (Nuromax) has a slow onset of action and long duration (>50 minute) of effect in man. Doxacurium may result in an increased propensity for histamine release and cardiovascular side effects when compared to other benzylisoquinolinium compounds. Work in dogs would suggest that 2.1 μg/kg IV approximates an ED_{50} while 3.5 μg/kg IV approximates an ED_{95} with duration of action at 108 ± 31 minutes (Martinez et al., 1996). Due to increased cost, minimal reported advantage, and lack of availability and clinical experience, atracurium is typically selected over other available benzylisoquinolinium compound for veterinary patients.

Aminosteroid Neuromuscular Blocking Agents

Pancuronium (Pavulon) was the first aminosteroid to be introduced as a NMB agent. The drug has a dose-dependent onset of approximately 5 minutes and long duration of action, up to 60 minutes in the dog (Gleed and Jones, 1982). Because repeated doses have a cumulative effect, constant-rate infusions are avoided. A large proportion of pancuronium is excreted by the kidney. The remainder is metabolized via the liver, making the duration of action increased in patients with renal and hepatic dysfunction. In addition to exerting its effect on postsynaptic nicotinic receptors at the NMJ, pancuronium also has a vagolytic effect by blocking cardiac muscarinic action, which may result in an increase in heart rate. Decreases to systemic vascular resistance, pulmonary vascular resistance, and coronary and renal artery dilation via prostaglandin I_2 release have also been noted in the dog (Hackett et al., 1989; Sai, 1998). In dogs, cats, pigs, and horses doses between 0.06 and 0.12 mg/kg IV have been reported (Gleed and Jones, 1982; Hildebrand et al., 1989; Miller et al., 1978; Veres-Nyéki et al., 2012). Pancuronium is stable at room temperature for 6 months.

Removal of a single positively charged methyl group from pancuronium creates vecuronium, an aminosteroid NMB agent that is essentially devoid of cardiovascular effects (Morris et al., 1983; Jones, 1985b). This molecular modification has several other important clinical implications, including molecular instability in solution which necessitates vecuronium be kept as a lyophilized powder and only reconstituted immediately prior to administration with stability of only 24 hours once reconstituted. Additionally, vecuronium has a shorter duration of action than pancuronium and an increase in lipid solubility, which results in greater biliary elimination than with pancuronium (Hill et al., 1994). Vecuronium has a dose-dependent onset of action of approximately 5 minutes and generally an intermediate duration of action of approximately 30 minutes, making its clinical utility similar to atracurium.

Vecuronium undergoes both hepatic metabolism, biliary clearance from the liver as parent compound, and about 25% undergoes renal elimination. Patients with renal or hepatic insufficiency may experience prolonged recovery if increased doses of vecuronium are administered. Dogs with diabetes mellitus have been shown to have a shorter duration of effect from vecuronium based upon both tactile train-of-four (TOF) and electromyography (Clark et al., 2012). The potency and duration of vecuronium does not, however, appear to be altered in dogs with autosomal recessive centronuclear myopathy (Martin-Flores et al., 2015b). In horses, it appears that a dose greater than 0.1 mg/kg IV would be needed for complete neuromuscular blockade and that this effect would have a uniquely long duration in this species, lasting over 120 minutes (Martin-Flores et al., 2012a, 2012b). Additionally, one case report documents that a dosage of 0.5 mg/kg IV edrophonium failed to completely reverse prolonged vecuronium-induced neuromuscular blockade in anesthetized dog as judged by peripheral nerve stimulation (Martin-Flores et al., 2011).

Rocuronium bromide (Zemuron), another member of the aminosteroid NMB agent family, lacks the acetyl ester that is found in the steroid nucleus of pancuronium and vecuronium and is less potent but similar in molecular weight to vecuronium. Rocuronium has a more rapid onset compared with atracurium and vecuronium, but similar duration of action (Carson et al., 1990; Gyermek et al., 2002). Additionally, rocuronium appears to be largely devoid of cardiovascular side effects, including no significant histamine release (Hudson et al., 1998). The drug is eliminated primarily via hepatic clearance while a small fraction is eliminated via the kidney. Detectable levels of rocuronium metabolites are not found. Rocuronium is stable for 60 days at room temperature. Rocuronium at clinically relevant concentrations may also bias the accuracy of lithium dilution cardiac output monitoring by interacting with the LiDCO sensor (Ambrisko et al., 2013).

In cats, a dosage of 0.6 mg/kg IV rocuronium had an onset time of 46 ± 11 seconds, produced no change to subject heart rate, and took 20.7 ± 5.4 minutes for TOF ratios to return to 0.9, a level consistent with full recovery from neuromuscular blockade (Auer and Mosing, 2006; McGrath and Hunter, 2006). Sixty seconds after rocuronium administration in cats, paralysis of the internal laryngeal muscles and conditions suitable for endotracheal intubation occurred that were comparable to topical lidocaine (Moreno-Sala et al., 2013). In dogs, rocuronium at 0.3 mg/kg and 0.6 mg/kg IV resulted in an onset of neuromuscular blockade in 2 ± 0.9 minutes and 1.1 ± 0.6 minutes with total recovery occurring at 23.8 ± 6.6 minutes and 31.9 ± 6.5 minutes respectively (Auer, 2007). Following a loading dose of 0.5 mg/kg IV, rocuronium was found to be suitable for constant-rate infusion at 0.2 mg/kg/h for up to 146 minutes (Alderson et al., 2007). Doses of rocuronium as low as 0.03–0.075 mg/kg IV in isoflurane-anesthetized dogs resulted in a centralized globe position and acceptable conditions for ophthalmic procedures (Briganti et al., 2015). In horses undergoing ophthalmic surgery, a dose of 0.3 mg/kg IV rocuronium produced effective neuromuscular blockade in 2.3 ± 2 minutes with a central globe position in 31 ± 2.8 seconds and a clinical duration of 32 ± 18.6 minutes in all 20 horses studied (Auer and Moens, 2011).

Asymmetric Mixed-onium Chlorofumarates

Gantacurium represents a new class of nondepolarizing NMB agents called asymmetric mixed-onium chlorofumarates, which are structurally distinct from the traditional aminosteroids and benzylisoquinolinium compounds. Gantacurium has an ultrashort duration of action and is degraded in plasma by pH sensitive chemical hydrolysis and inactivation by cysteine adduction. It does not undergo Hofmann elimination. In dogs anesthetized with thiopental, nitrous oxide, and isoflurane, the ED_{95} was 0.06 mg/kg IV with an onset time of 107 seconds and duration of action of 5.2 minutes (Heerdt et al., 2004). In cats following dexmedetomidine and propofol, IV gantacurium at 0.5 mg/kg abolished laryngospasm in 100% of cats and induced apnea for 3 ± 1.5 minutes (Martin-Flores et al., 2015a). At clinical doses, cats and dogs do not appear to have appreciable cardiovascular effects; however, in humans, transient cardiovascular side effects were observed at doses beginning at three times the ED_{95} and were consistent with histamine release (Belmont et al., 2004). Recovery is accelerated with AChE inhibitors such as edrophonium. In humans, exogenous administration of cysteine can also accelerate the antagonism of gantacurium-induced neuromuscular blockade. Gantacurium is not stable in aqueous solution and, like vecuronium, is provided as a lyophilized powder that is reconstituted prior to administration.

Trisquaternary Ether Neuromuscular Blocking Agents

Two drugs are represented in this class of compounds that currently have no clinic significance for use in North America, primarily due either to significant side effects or lack of a currently available marketed product. Gallamine

(Flaxedil) is a NMB agent first developed in 1947 (Rhagavendra, 2002). Its desirable qualities were rapid onset of action (3 minutes) and intermediate to long duration of action in the range of 40 minutes. Gallamine has significant parasympatholytic activity and is associated with tachycardia and hypertension (Lee, 2001; Clark and Mitchelson, 1976). Gallamine can also cause histamine release. Approximately 1 mg/kg gallamine causes complete muscle paralysis in both dogs and cats within 1–2 minutes after IV injection and lasts 15–20 minutes. A hypotensive response may be induced in cats with gallamine but is infrequently observed in dogs.

Alcuronium (Alloferin) is another trisquaternary ether NMB agent whose profile is distinguished by a long duration of effect, rendering it generally less desirable than the short to intermediate acting agents (Diefenbach et al., 1995). The mean duration of effect for alcuronium reported in dogs is 70 minutes (Jones et al., 1978). This agent relies entirely on renal elimination for its clearance, undergoing no biodegradation, and relevant concentrations of alcuronium can still be detected in the plasma up to 12 hours postinitial administration of neuromuscular blockade (Diefenbach et al., 1995). This agent is not marketed for use within the United States.

Depolarizing Agents

Succinylcholine chloride, USP (Quelicin, Anectine, Sucostrin, Suxamethonium), and decamethonium bromide, USP (Syncurine, C-10), are members of the depolarizing group of NMB agents. These drugs exert their paralytic effects by interfering with ACh-mediated depolarization of the postsynaptic membrane. In contrast to the well-defined mechanism of the competitive agents, certain aspects of the mechanism(s) of depolarizing neuromuscular blockers are continually debated.

Only succinylcholine is currently available for use within veterinary medicine as a depolarizing NMB agent. However, its utility has dwindled and even this agent has largely been replaced by newer nondepolarizing NMB agents because of succinylcholine's side-effect profile and potential patient monitoring challenges. Succinylcholine is composed of two molecules of ACh linked back to back through acetate methyl groups. Succinylcholine, like ACh, stimulates cholinergic receptors at the NMJ and at nicotinic ganglionic and muscarinic autonomic sites, ultimately opening the ion channel at the nicotinic cholinergic receptor. Succinylcholine elicits a prolonged depolarization of the neuromuscular end-plate that does not allow the postsynaptic membrane to completely repolarize and therefore renders the neuromuscular motor end-plate unresponsive to the normal action of ACh. Because of the initial stimulatory depolarizing action, transient contraction of muscle cells occurs after administration of succinylcholine. Clinically, this is characterized in the intact animal by asynchronous muscular contractions of the head, body trunk, and limbs. Fasciculations are not always apparent in anesthetized animals. Because of the persistent depolarization of the postsynaptic nicotinic membrane, continued impulse transmissions are blocked and a flaccid paralysis ultimately ensues. The mechanism of depolarizing NMB agent action has historically been described as biphasic.

Phase I block: Initially a phase I block results from depolarization of the neuromuscular motor end-plate region by succinylcholine resulting in an increased and persistent permeability of the postsynaptic membrane to sodium and potassium ions, specifically an influx of sodium ions into the cell and an efflux of potassium ions out of the cell. The ion channel pores subsequently close and become inactivated. As a result, ACh cannot act as a neurotransmitter, and impulse transmission to evoke a muscle response fails. The membrane potential must be reset in order for the channel to be reactivated again. When Ach causes depolarization it is immediately hydrolyzed by AChE within the synaptic cleft and its effect is very short lived. Succinylcholine causes a persistent alteration in membrane potential because it is not hydrolyzed but must slowly diffuse away from the NMJ and be metabolized by plasma cholinesterases. It should be pointed out that ACh, when in excess, also causes persistent depolarization block of cholinergic synaptic junctions (Appiah-Ankam and Hunter, 2004).

Phase II block: Phase II block occurs in some instances after prolonged exposure or large doses of a depolarizing agent and motor end-plate sensitization and is characterized by a change from the depolarizing block to one that more closely resembles the effect of nondepolarizing NMB agents. The actual mechanisms involved are poorly understood and opinion is contradictory as to this transition. Zaimis (1959) believes, for example, that confusion has occurred because in some species some blocking agents have a "dual mechanism"; that is they cause some effects that resemble depolarization block and cause other effects that resemble competitive blockade (Galindo and Kenney, 1974).

After exposure of isolated nerve-muscle preparations to succinylcholine, the initial peak level of depolarization subsides. Subsequently, the end-plate becomes transiently sensitive to depolarizing agents. Gradually, a competitive-like blockade results and seems to be at least partially susceptible to reversal by cholinesterase inhibitors. Though the overall importance of phase II block has not been clearly defined for depolarizing NMB agents, its potential for occurrence and recognition is important.

As a group, the depolarizing NMB agents cause depolarization of receptor areas of muscle fibers sometime during their course of action. In general, unlike nondepolarizing NMB agents, administration of an AChE inhibitor (e.g., edrophonium, neostigmine) will not reverse the effects of depolarizing NMB agents such as succinylcholine and may in fact delay recovery.

Succinylcholine has a rapid onset of effect and an ultrashort duration of action, which has made the agent historically ideal to assist in rapid human endotracheal intubation. The short duration of action (approximately 6–10 minutes) of succinylcholine is due to its rapid hydrolysis by plasma cholinesterase (psuedocholinesterase, butyrlcholinesterase) to succinylmonocholine and choline. Because there is limited plasma cholinesterase activity at the NMJ, the neuromuscular blockade that occurs from succinylcholine is terminated by the drug's diffusion away from the NMJ back into the circulation. This has important clinical consequences in that the drug is not reversible by AChE inhibitors and factors that decrease plasma cholinesterase activity may prolong recovery. Common factors seen in veterinary patients that may decrease plasma cholinesterase activity include hepatic disease, advanced age, malnutrition, neoplasia, pregnancy, burns, monoamine oxidase inhibitors, terbutaline, esmolol, and metoclopramide (Birch et al., 1956; Lepage et al., 1985; Kao et al., 1990; Barabas et al., 1986).

Hansson (1956) reported that the IV ED_{50} (dose that reduced muscle twitch by 50%) of succinylcholine in the sciatic nerve-gastrocnemius muscle preparation of anesthetized dogs was 0.045–0.060 mg/kg. This dose did not effectively paralyze the respiratory muscles, however, and 0.085 mg/kg was required to induce transient apnea, whereas 0.11 mg/kg and 0.22 mg/kg were needed to cause apnea for 18–21 minutes and 23–27 minutes, respectively. In unanesthetized dogs, IM administration of 0.12 mg/kg succinylcholine caused ataxia in 5 minutes and forced abdominal respiration in 7 minutes; recovery was apparently complete in 30 minutes. In clinical situations, 0.3 mg/kg succinylcholine administered intravenously will usually afford good muscle relaxation in dogs, whereas in the cat, 1 mg/kg may be required. In dogs, Hansson (1956) reported that 0.15 mg/kg succinylcholine was effective in paralyzing the diaphragm during thoracotomy procedures. However, Eyster and Evans (1974) suggested the use of 0.5 mg/kg succinylcholine for muscle relaxation in dogs during thoracotomy for open-heart surgery. This dose was also reported to control muscle twitches evoked by inadvertent stimulation of nerves during use of electrocautery. Duration of paralysis varies and should be closely monitored.

In rhesus monkeys, 1–2 mg/kg succinylcholine administered intravenously has been used for restraint for tuberculosis testing and endotracheal intubation (Lindquist and Lau, 1973). In pigs, approximately 2 mg/kg succinylcholine is effective. Much smaller amounts (0.01–0.02 mg/kg) are required in cattle and sheep. Hansson (1956) reported that 0.13–0.18 mg/kg succinylcholine is required to immobilize unanesthetized horses. However, the generally accepted dose of succinylcholine in horses, when used alone, is 0.088 mg/kg (Lumb and Jones, 1973).

Succinylcholine historically has been used without anesthesia in horses for casting and restraint during brief surgical procedures such as castration. This practice is not condoned, because no anesthesia is afforded for painful procedures, severe fright is evoked, and pronounced cardiovascular disturbances and even myocardial damage may result. Succinylcholine should not be used as a sole restraining agent during surgical procedures but only in conjunction with a general or local anesthetic.

Frequent side effects from succinylcholine have resulted in the drug largely being replaced in veterinary medicine by newer agents. Succinylcholine stimulates all cholinergic autonomic receptors, including nicotinic receptors on both sympathetic and parasympathetic ganglia and muscarinic receptors in the sinus node of the heart (Galindo and Davis, 1962). As a prominent clinical manifestation of generalized autonomic stimulation, cardiac dysrhythmias, including sinus bradycardia, junctional ectopy, and ventricular dysrhythmia, may occur. At lower doses negative chronotropic effects predominate and at higher doses tachycardia may occur.

In humans, the administration of succinylcholine to an otherwise healthy patient raises plasma potassium levels by approximately 0.5 mEq/l. This phenomenon results because with channel activation by acetylcholine, sodium moves into the cells, and potassium exits to the extracellular space. This mild increase in potassium is generally well tolerated in healthy patients and does not result in arrhythmias. However, in patients with preexisting hyperkalemia the additional rise in plasma potassium levels could be of clinical concern. The combination of severe metabolic acidemia and hypovolemia has, in both rabbits and man resulted, in severe and life threatening hyperkalemia (Antognini and Gronert, 1993; Schwartz et al., 1992).

Succinylcholine may cause a rise in intraocular pressure (IOP). The mechanism by which the drug increases IOP is not known but may involve contraction of tonic myofibrils or choroidal blood vessel dilation or both. In man the increased IOP occurs within 1 minute after injection, peaks between 2 and 4 minutes, and subsides by 6 minutes (Pandey et al., 1972). Succinylcholine is also known to increase intragastric pressure, presumed to be due to fasciculation of abdominal skeletal muscles. Succinylcholine can be a potential trigger for malignant hyperthermia in susceptible patients and

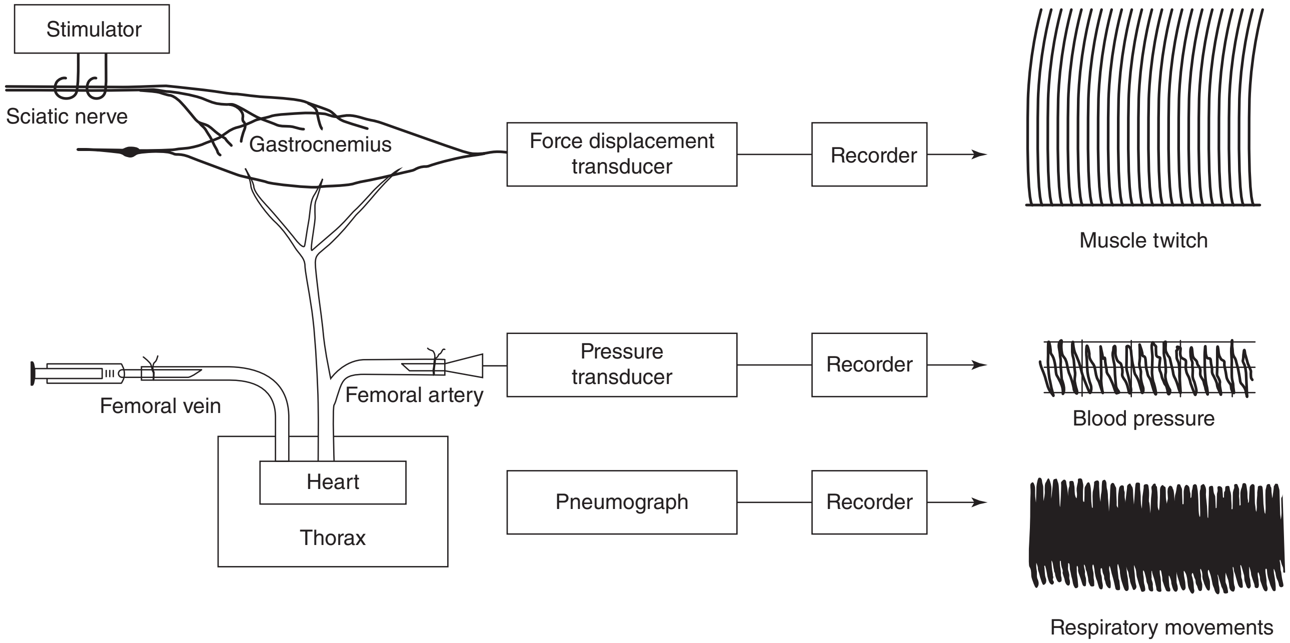

Figure 10.4 Schematic representation of a sciatic nerve-gastrocnemius muscle preparation in an anesthetized cat. Stimulation of the isolated and decentralized sciatic nerve evokes contraction (muscle twitch) of the gastrocnemius muscle. Femoral arterial blood pressure and respiratory movements can be measured concurrently. Neuromuscular blocking drugs can be administered intravenously and changes in muscle twitch height observed.

produce malignant hyperthermia-like episodes, and has the potential to increase intracranial pressure (Minton et al., 1986). Muscle pain, increases to serum creatine kinase, and myoglobinemia have all been reported in man following the drug's administration (Brodsky et al., 1979; McLoughlin et al., 1992).

Pharmacological Effects of Neuromuscular Blocking Agents

When administered, NMB agents are given only to anesthetized patients to provide relaxation of skeletal muscles. It should be remembered that NMB agents do not possess analgesic or amnestic properties and other drugs must be utilized for these effects. Because of a narrow therapeutic index, the clinical usage of NMB agents necessitates strict oxygen supplementation and assisted ventilation in conjunction with qualified supervision by experienced personnel. Said personnel must be thoroughly familiar with the indications, limitations, and adverse effects of NMB agents, principles of monitoring, as well as familiarity with the use of their reversal agents.

Skeletal Muscle

Competitive Neuromuscular Blocking Agents

Nondepolarizing curare-like drugs interact with nicotinic cholinergic receptors of skeletal muscle cells and render them insensitive to the transmitter function of Ach in a dose-dependent fashion. Flaccid paralysis may ultimately occur. Neither axonal conductance nor response to direct stimulation of muscle is blocked by nondepolarizing NMB agents.

A schematic representation of an in vivo nerve-muscle preparation is shown in Figure 10.4. This sciatic nerve-gastrocnemius muscle preparation has been used to examine the actions and pharmacological interactions of NMB agents. In this preparation, electrical stimulation of the sciatic nerve causes contraction (kg of isometric tension) of the gastrocnemius muscle.

Figure 10.5 demonstrates the neuromuscular blocking effect of *d*-tubocurarine on the stimulated muscle twitch of the sciatic nerve-gastrocnemius muscle preparation of a cat, as described in Figure 10.4. Provided a consistent supramaximal stimulus is delivered at a frequency of no greater than once every 7–10 seconds, a depression in muscle twitch response is not seen until approximately 75% of nicotinic cholinergic receptors are blocked. Loss of muscle twitch in response to electrical stimulation requires 90–95% blockade of cholinergic receptors on the motor end-plate (Kelly and Brull, 1993). In this example, muscle twitch quickly decreases after IV injection of *d*-tubocurarine, reaches peak depression within a few minutes, and then gradually returns to normal in approximately 15 minutes. Tubocurarine does not evoke an initial increase in muscle twitch; this lack of facilitation is a consistent finding with nondepolarizing agents.

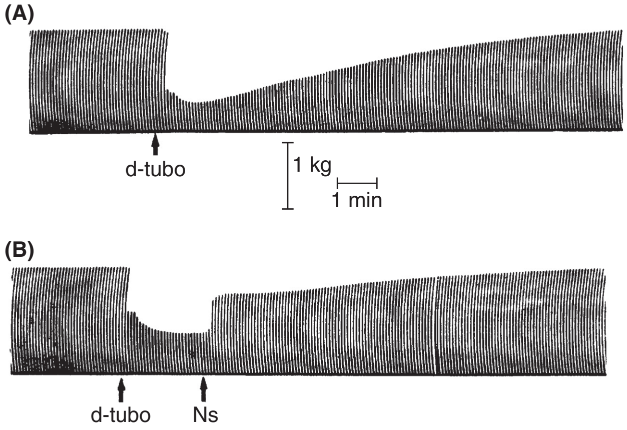

Figure 10.5 Neuromuscular blocking effect of *d*-tubocurarine (*d*-tubo) and reversal of the *d*-tubo by neostigmine (Ns) in a sciatic nerve-gastrocnemius muscle preparation of a cat. The cat was anesthetized with pentobarbital; muscle twitch was monitored as described in Figure 10.4. (**A**) Typical depression of muscle twitch by *d*-tubo (0.2 mg/kg) administered intravenously at designated arrow. (**B**) Antagonism of *d*-tubo (0.2 mg/kg) induced depression of muscle twitch by Ns (0.1 mg/kg). Agents were administered intravenously at arrow. Notice rapid antagonism of the neuromuscular blocking effect of *d*-tubo by Ns. Compare this with the lack of antagonism by Ns of the muscle twitch depressant effect of succinylcholine in Figure 10.6.

Antagonism of the neuromuscular blocking effects of *d*-tubocurarine by administration of a cholinesterase inhibitor, neostigmine, is shown in Figure 10.5. By comparing the two tracings in this figure, it is readily apparent that neostigmine markedly hastens recovery from muscle twitch depression caused by *d*-tubocurarine. This antagonistic interaction is primarily attributed to the anticholinesterase activity of neostigmine. Inhibition of cholinesterase delays the catabolic breakdown of ACh and allows its accumulation at receptor sites. Newly available ACh, now in increased concentration at the postsynaptic membrane, effectively competes with *d*-tubocurarine for the cholinergic receptors. ACh-mediated depolarization of the end-plate, muscle action potentials, and muscle contraction are restored; muscle twitch quickly returns to normal.

The sensitivity to the range of receptor blockade represented in the single twitch muscle preparation of Figure 10.4 is narrow and therefore limits its clinical usefulness as a measure of receptor blockade. In clinical situations, a TOF stimulation pattern is often used to determine the extent of blockade present when NMB agents are being used. Four supramaximal stimuli are delivered at a frequency of 2 Hz and, in normal muscle, four clearly separated twitch responses can be observed in the muscle, which ideally are quantified in amplitude via the use of mechanomyography or acceleromyography (Kelly and Brull, 1993). After administration of a nondepolarizing NMB agent, the TOF stimulation pattern will elicit muscle responses that exhibit fade. The degree of fade is proportional to the extent of receptor blockade. The amplitude of the fourth twitch response (T4) compared to the amplitude of the first twitch response (T1) gives a T4/T1 ratio or TOF ratio. With no blockade the TOF ratio is 1.0. The T4 amplitude will start to decrease when 70–75% of receptors are blocked by a nondepolarizing NMB agent. If the T4 response is lost completely, approximately 80% of the motor end-plate cholinergic receptors are blocked. If T3 and T2 responses disappear, this represents 85% and then 85–90% of receptor occupancy. When 90–95% of receptors are occupied at the NMJ then T1 through T4 will have disappeared (Kelly and Brull, 1993). In order to assess recovery from neuromuscular blockade, a TOF ratio of 0.9 should be achieved before an animal is deemed suitably recovered to be extubated (Ali et al., 1970). Reversal of a nondepolarizing NMB agent can be safely administered when a TOF count is at least demonstrating two twitches or greater (Viby-Mogenson, 2000; Jones et al., 2015).

Depolarizing Neuromuscular Blocking Agents

Succinylcholine elicits transient muscle fasciculations prior to causing neuromuscular paralysis, due to initial depolarization of the motor end-plate and is characterized in the intact animal by asynchronous muscular contractions of the head, body trunk, and limbs.

The in vivo neuromuscular blocking effect of a small dose of succinylcholine in a cat nerve-muscle preparation is shown in Figure 10.6. Initially, there is a slight and transient facilitatory effect of succinylcholine on neuromuscular transmission; muscle twitch height momentarily increases by a small increment as a result of the initial depolarizing effect of the drug. Subsequently, however, muscle twitch rapidly decreases and within 1–2 minutes maximum depressant effect is obtained. Shortly thereafter, the neuromuscular effects of succinylcholine subside and muscle twitch returns to normal within an additional 5–8 minutes. The magnitude and duration of neuromuscular paralysis is dependent upon the dosage of succinylcholine. The relatively short duration of succinylcholine activity is from rapid biotransformation of this drug by plasma cholinesterase.

The effects of a cholinesterase inhibitor, neostigmine, on the neuromuscular paralysis produced by succinylcholine are demonstrated in Figure 10.6. By comparing the two tracings in this figure, it is apparent that neostigmine potentiated the muscle twitch depression evoked by succinylcholine and prolonged recovery from the effects of this agent. This synergistic interaction is primarily attributed to the anticholinesterase activity of neostigmine, resulting in decreased biotransformation of both succinylcholine and endogenous ACh. Thus succinylcholine and ACh are available at receptor sites for

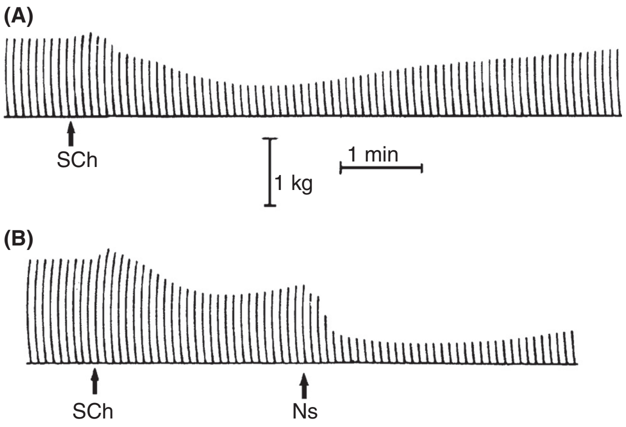

Figure 10.6 Neuromuscular blocking effect of succinylcholine (SCh) and augmentation of its effect by neostigmine (Ns) in a sciatic nerve-gastrocnemius muscle preparation of a cat. The cat was anesthetized with pentobarbital; muscle twitch was monitored as described in Figure 10.4. (**A**) Typical depression of muscle twitch by SCh (0.04 mg/kg) administered intravenously at designated arrow. (**B**) Augmentation of the muscle twitch depressant effect of SCh (0.04 mg/kg) by Ns (0.1 mg/kg). Agents were administered intravenously at designated arrows. Notice augmentation of the degree and duration of effect of SCh by Ns. Compare this with the antagonism by Ns of the neuromuscular blocking effect of *d*-tubo in Figure 10.5.

longer periods and the duration of depolarizing neuromuscular paralysis is prolonged.

The potency of neuromuscular effects of succinylcholine varies in different species (Hansson, 1958), as shown schematically in Figure 10.7, with bovine and canine species being quite sensitive to succinylcholine, whereas horses and pigs are considerably less responsive. This difference is probably dependent upon species differences in the activity of plasma cholinesterase, the enzyme that biotransforms succinylcholine (Radeleff and Woodard, 1956; Palmer et al., 1965). Cattle and sheep, for example, have considerably less detectable plasma cholinesterase activity than horses and pigs. Administration of purified cholinesterase preparation to dogs increases resistance to succinylcholine (Hall et al., 1953).

Autonomic Effects

Synaptic transmission at autonomic ganglia involves activation by ACh of nicotinic receptors of the postganglionic nerve body (see Chapter 8). It is not surprising, therefore, that NMB agents may also alter ganglionic transmission.

Tubocurarine is an excellent example of a drug selected for site of action at nicotinic receptors of the somatic myoneural junction that as a side effect also acts at ganglionic nicotinic receptors. Tubocurarine interacts with ganglionic receptors, renders them inaccessible to ACh, and thereby increases the threshold of the postganglionic nerve to ACh. However, as a general rule, autonomic ganglia are less sensitive to curare than are the myoneural junctions. Ganglionic impulse transmission involves, at least partially, a muscarinic pathway (see Chapter 7); *d*-tubocurarine has little blocking effect on muscarinic receptors. Thus in most cases it would be anticipated that ganglionic transmission is functional during treatment with curare-like drugs. Nevertheless, hypotension believed to be partly dependent upon ganglionic blockade can occur after administration of *d*-tubocurarine.

Other NMB drugs, both competitive and depolarizing types, have been shown experimentally to alter ganglionic transmission, but in clinically insignificant amounts. Succinylcholine induces transient ganglionic stimulation prior to blockade evoked by larger doses. The former effect may partially explain hypertension that has occurred subsequent to succinylcholine administration.

Parasympathetic effects of NMB agents are usually minimal. Succinylcholine is approximately 1,000 times less potent than ACh in eliciting contraction of guinea pig ileum. In dogs, large doses of succinylcholine induce

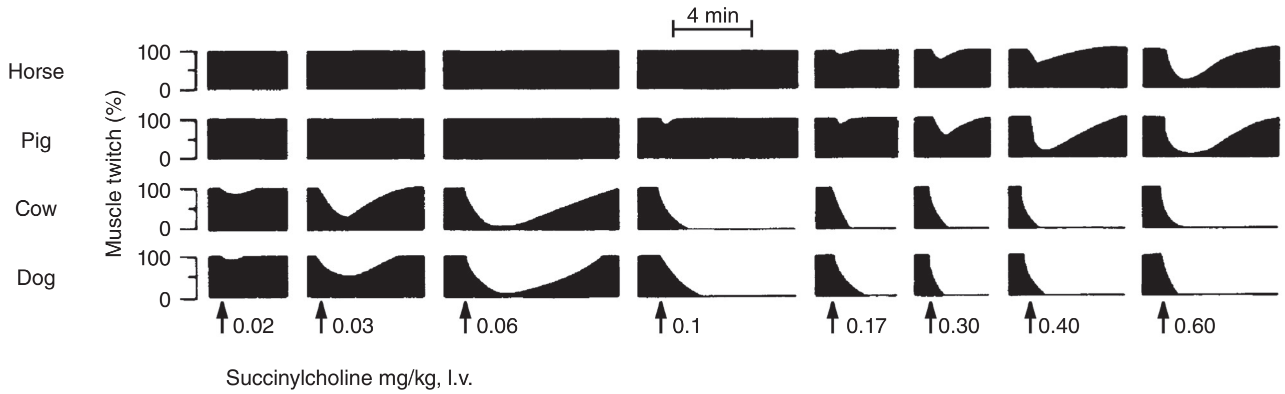

Figure 10.7 Schematic representations of the neuromuscular blocking effect of succinylcholine iodide in nerve-muscle preparations of different species during barbiturate anesthesia. Notice interspecies differences in degree and duration of paralysis caused by succinylcholine. Source: Modified from Hansson 1956, Lumb and Jones 1973.

salivation; this is antagonized by pretreatment with atropine (Hansson, 1956).

Histamine Release

d-Tubocurarine causes release of histamine. The magnitude of this response varies, depending on species, dosage, and rate and route of administration. Histamine-like wheals can be produced by subdermal and intraarterial administration of *d*-tubocurarine. In vivo, increased respiratory tract secretions and bronchospasm seen after administration of *d*-tubocurarine have been attributed to histamine release, as has the hypotensive effect of *d*-tubocurarine. Pretreatment with antihistamine drugs antagonizes these side effects; they are not inhibited by atropine or neostigmine. Succinylcholine and gallamine are very weak histamine-releasing agents.

Central Nervous System

Although synaptic transmission in the brain is altered by direct application of NMB drugs into the brain, CNS effects are nondetectable when these drugs are administered by other routes. NMB agents do not gain entry into the CNS to any appreciable extent because of the presence of the highly charged quaternary ammonium moieties. Therefore, neither CNS depression nor tranquilization is produced by NMB agents. Nonambulation results only from peripheral myoneural paralysis. This was decisively confirmed when Smith (Smith et al., 1947) allowed himself to be paralyzed with *d*-tubocurarine. At no time during the experiment did he experience hypnosis, tranquilization, amnesia, anesthesia, or analgesia. He simply could not voluntarily breathe or move, an experience described as quite frightful.

Cardiovascular Effects

As outlined above, *d*-tubocurarine often induces hypotension, particularly if rapidly administered to dogs. Slight increases in heart rate and cardiac output have been observed after administration of gallamine, apparently from a vagolytic effect on the heart (Longnecker et al., 1973). Others have reported no significant cardiovascular changes after IV administration of gallamine to anesthetized dogs (Evans et al., 1977). In cats, mild atropine-like effects on the heart were observed after injection of gallamine, pancuronium, and alcuronium (Hughes and Chapple, 1976).

Studies with pancuronium in humans and dogs indicated that this agent evokes slight increases in heart rate, blood pressure, and cardiac output during thiobarbiturate anesthesia (Coleman et al., 1972; Reitan and Warpinski, 1975). Cardiovascular effects of this agent were absent if patients were pretreated with atropine. Others have reported no significant cardiovascular changes with pancuronium (Brown et al., 1973). Similarly, studies have indicated that neither pancuronium nor gallamine significantly altered heart rate or blood pressure in anesthetized horses (Klein et al., 1983). Atracurium (up to 0.6 mg/kg) and vecuronium (up to 0.2 mg/kg) were reported to have negligible effects on arterial blood pressure in dogs (Jones, 1985a).

Succinylcholine usually evokes minimal cardiovascular changes in horses or dogs if administered during general anesthesia; blood pressure remains fairly constant if mechanical ventilation is provided (Evans et al., 1977; Benson et al., 1979).

Subparalytic doses of succinylcholine increase the arrhythmogenicity of epinephrine during light halothane anesthesia in dogs (Tucker and Munson, 1975). In dogs not treated with succinylcholine, an average dose of 4.15 μg/kg epinephrine was required to evoke premature ventricular contractions, whereas an average dose of 1.6 μg/kg epinephrine was the arrhythmogenic dose in dogs pretreated with 0.25 mg/kg succinylcholine. However, *d*-tubocurarine provides a slight protection against epinephrine-induced arrhythmias. Mechanisms involved in these drug interactions have not been clarified. If deemed essential, catecholamines should be used cautiously in patients treated with depolarizing NMB agents. Also, succinylcholine has been reported to increase susceptibility to the myocardial irritant effects of digitalis preparations, and it has been suggested that succinylcholine may be contraindicated in digitalized patients (Dowdy et al., 1965).

Pronounced cardiovascular side effects have been reported in horses after administration of succinylcholine (Larson et al., 1959; Hofmeyer, 1960; Lees and Tavernor, 1969). In general, these effects seem to be more pronounced in unanesthetized and unsedated animals than during general anesthesia. Severe hypertension, initial bradycardia followed by tachycardia, atrioventricular conduction disturbances, and extrasystoles have been reported, and myocardial damage has been suspected. Early institution of positive pressure ventilation has been reported to block the blood pressure effect. The hypertensive response seems to be at least partially mediated by the succinylcholine-induced dyspnea and the accompanying arterial $\mathrm{Po_2}$–$\mathrm{Pco_2}$ disturbances, causing a reflexogenic increase in blood pressure. Direct activation of autonomic ganglia by succinylcholine may also be involved.

It should be remembered that NMB agents do not depress the brain unless or until apnea-induced hypoxia actually causes syncope. Prior to hypoxic states, skeletal muscle paralysis affords no depression whatsoever of conscious centers of the brain of unanesthetized animals. It seems likely, then, that the novel sensations experienced by conscious animals as they are being paralyzed evoke profound fright. This can cause activation of autonomic centers within the brain. Autonomic discharge may be altered markedly resulting in cardiovascular side effects. Autonomic blockade has been shown to substantially decrease the cardiovascular side effects of succinylcholine, supporting the argument for its association with a stress response in those animals.

Ocular Effects

Clinically important ocular effects may occur as a result of pronounced contracture of ocular muscles that occurs after treatment with depolarizing neuromuscular blockers. These agents are contraindicated in glaucoma, since intraocular pressure may be increased. There is no contraindication for nondepolarizing NMB agents in this instance.

Serum Potassium

Depolarizing NMB agents cause a release of K^+ from skeletal muscle. This change is typically small in magnitude, but could be of concern in patients that already have an elevation in serum potassium. Elevation of serum K^+ may be particularly significant, if repeated injections of the NMB are given. There could be a greater risk in animals with muscle disorders, such as Golden Retriever muscular dystrophy (Larach, 1997).

Drug Interactions

Various drugs influence the pharmacological effects of muscle relaxants. NMB agents themselves alter activity of other neuromuscular agents. As would be expected, competitive agents summate with each other. Similarly, depolarizing agents also interact synergistically with one another. However, tubocurarine decreases the muscle twitch depressant effects of succinylcholine. This is related to persistent occupation of a certain portion of receptors by tubocurarine, although muscle twitch may have recovered (see Section Margin of Safety of Neuromuscular Transmission). Depolarization of the end-plate by succinylcholine is partially impeded by the stabilizing effects of tubocurarine. Succinylcholine antagonizes the effects of curare as a result of the partial agonistic characteristics of the former agent. These complex antagonistic interactions, however, have no clinical application, since they depend upon complicated treatment and time and dosage schedules. During clinical situations, NMB agents should not be used in attempts to reverse the effects of other types of NMB agents, since potentiation may occur despite experimental results to the contrary.

The interaction of cholinesterase inhibitors with NMB agents has been discussed in Section Skeletal Muscle. Cholinesterase inhibitors decrease responsiveness to the competitive agents, while they tend to increase intensity and duration of action of depolarizing agents (Sunew and Hicks, 1978). Organophosphate pesticides and anthelmintics, carbamates, and any other type of cholinesterase inhibitor will cause similar interactions.

Aminoglycoside antibiotics (neomycin, streptomycin, dihydrostreptomycin, kanamycin, gentamicin) decrease the release of ACh from the nerve and also the sensitivity of the end-plate to ACh (Pittinger and Adamson, 1972; Adams et al., 1976a). They do not cause depolarization. Their effects in many ways resemble those of low Ca^{++} or excess Mg^{++}. The presynaptic effect of antibiotics is believed to be due to interruption of Ca^{++} -dependent events at the axonal membrane (Adams, 1984). Cholinesterase inhibitors such as neostigmine antagonize the postsynaptic depressant effect of these antibiotics. Calcium antagonizes the presynaptic action and is usually more effective than neostigmine in reversing the neuromuscular paralyzing effects of aminoglycoside antibiotics. These antibiotics interact synergistically at the myoneural junction with NMB agents, anesthetics, and other antibiotics. The clinical significance of neuromuscular interactions of antibiotics and other drugs has been well established in humans and has been suggested in other species (Adams and Bingham, 1971). These subjects have been reviewed (Pittinger et al., 1970; Adams et al., 1976b; Keller et al., 1992).

Different disease states influence pharmacological effects of NMB agents. Hepatic synthesis of cholinesterase is decreased in the presence of liver disease. The duration of succinylcholine activity may be prolonged if the liver is seriously affected. Impairment of hepatic biotransformation may also cause prolongation of the effect of vecuronium. Renal dysfunction can delay excretion of *d*-tubocurarine, gallamine, doxacurium, and pancuronium.

Clinical Use

Muscle paralysis proceeds at different rates in different body regions after administration of a NMB agent. Usually, the extraocular muscles, the facial muscles, and those of the head and neck are affected first, often within 0.25–1 minute after injection. The tail is usually affected with the head and neck. Subsequently, muscles of the limbs are paralyzed, then the deglutition and laryngeal muscles (glottis). Abdominal muscles, intercostal muscles, and the diaphragm are then paralyzed in this order. Recovery usually proceeds in the reverse of this sequence (Hall, 1971).

Attempts have been made in clinical practice to use the sequential development of muscle paralysis by administering doses of NMB agents adequate to paralyze ambulatory muscle but insufficient to affect the diaphragm. This rarely has proven to be effective, because respiratory insufficiency may still occur, although the diaphragm is seemingly spared. Therefore, it is imperative that apparatus for administering mechanical ventilation be available when NMB agents are used clinically. To circumvent the need for immediate establishment of an adequate airway and other emergency procedures, it is wise to routinely perform tracheal intubation and institute ventilation whenever a NMB agent is used.

Muscle relaxants have been used in clinical practice for several purposes: to facilitate tracheal intubation; to paralyze respiratory muscles so that mechanical ventilation can be easily controlled; to increase muscle relaxation to facilitate surgical access to difficult anatomic regions; to evoke muscle relaxation to facilitate orthopedic manipulations or improve ocular positioning for surgery and as part of balanced anesthesia procedures to reduce the amount of general anesthetic required.

Tracheal intubation may be performed in a unanesthetized animal immediately after a paralyzing dose of NMB agent has taken effect. Prior administration of sufficient sedation or tranquilization is advisable for humane reasons and to circumvent potential side effects that may be precipitated by fear reaction to paralysis.

A wide range of dosages of NMB agents have been reported for use of these drugs during anesthesia (Hansson, 1956; Tavernor, 1971; Lumb and Jones, 1973). Often this variance reflects differences in the procedures reported in the original studies, for example, the use of different anesthetics and sedatives, different salts of the NMB agent, and in some cases the use of unanesthetized subjects. NMB agents should be given to effect rather than by bolus administration of a set precalculated dose. It is advisable for these drugs to be administered by titration during anesthesia and to be continuously correlated with the extent of muscle paralysis in the patient. The use of neuromuscular monitoring techniques markedly improves the ability to accurately dose and safely terminate the effects of neuromuscular blockade in all animals. Care should always be taken during the use of NMB agents to ensure that the animal does not suffer from residual paralysis as it enters the recovery period postanesthesia.

Margin of Safety of Neuromuscular Transmission

The concept of a margin of safety of neuromuscular transmission bears discussion in relation to clinical use of these drugs. It has been estimated that a relatively large percentage of the cholinergic receptors must be occupied by a paralyzing agent before muscle twitch fails. In the cat diaphragm, for example, muscle twitch is not affected until about 80% of the receptors are blocked by *d*-tubocurarine, and twitch is not completely abolished until about 90% of the receptors are occupied (Waud and Waud, 1972). Accordingly, for recovery of the diaphragm from the effects of a previous injection of *d*-tubocurarine, only a small percentage (5% in dogs, 18% in cats) of the receptors need to be free. Therefore, and most important, although to all outward signs recovery seems complete, over 80% of the receptors can still be blocked.

Recognition of this aspect becomes clinically important in the postoperative recovery room and should be considered in patients that have been exposed to NMB drugs and/or other myoneural depressants such as anesthetics. As a patient regains some control of voluntary muscles, spontaneous respiration returns and may seem completely normal. However, it must be remembered that at this time an extremely small margin of safety of neuromuscular transmission exists. That is, only a small percentage of the postsynaptic receptors are available for interaction with ACh; this small fraction of receptors is now responsible for maintaining muscle contraction. Therefore, if the patient is then exposed to another drug that as a side effect depresses neuromuscular function (even though it may be minimal or even nondetectable normally), disastrous complications may result. Anesthetic mortality has occurred in humans that can be attributed to such interactions. For example, Pridgen (1956) reported the anesthetic deaths of two children who were given neomycin immediately after completion of successful laparotomies under ether anesthesia. Initially, respiration was adequate, but within a short time after administration of the antibiotic, persistent apnea occurred. Death followed several hours later. It seems likely that the margin of safety of neuromuscular transmission was reduced in these infants by ether, resulting in marked augmentation of the neuromuscular blocking properties of neomycin. Pittinger et al. (1970) estimated a 9% death rate in human patients experiencing antibiotic-induced respiratory problems in conjunction with anesthetics and NMB agents. Apnea and eventual death in a traumatized dog were attributed to antibiotic (dihydrostreptomycin)-induced neuromuscular paralysis (Adams and Bingham, 1971).

These examples illustrate potential problems that may be inadvertently introduced in a patient that seemingly is recovering quite well from anesthesia and surgery. The margin of safety of neuromuscular transmission should be considered any time that anesthetics, NMB agents, or any other drug that depresses myoneural function are used in multiple drug regimens.

Clinical Reversal of Neuromuscular Paralysis

Treatment of persistent neuromuscular paralysis and/or treatment of inadvertent overdosage of NMB agents should be approached conservatively (Bevan et al., 1992).

The first step should be to initiate immediate (or to continue) positive pressure ventilation and to withdraw administration of the involved NMB agent. Often, ventilation will allow adequate time for the drug to be disposed of or metabolized by the patient's system. Exposure to other drugs that may synergistically interact with NMB agents should be avoided. However, if the effect of a neuromuscular blocker is no longer needed as the end of a procedure is approaching, reversal of the neuromuscular blockade from a nondepolarizing NMB agent is a good option. There are two classes of drugs used for reversal of neuromuscular blockade: the AChE inhibitors and the cyclodextrin, suggamedex (Jones et al., 2015).

Acetylcholinesterase Inhibitors

Acetylcholinesterase inhibitors, including edrophonium, neostigmine, and pyridostigmine, are available to reverse the action of nondepolarizing NMB drugs. As discussed in previous sections, AChE inhibitors are not routinely effective for the reversal of depolarizing NMB agents such as succinylcholine unless prolonged administration or repeat dosages have resulted in phase II block. Fortunately, recovery from succinylcholine is rapid and spontaneous because of plasma cholinesterases and the issue of drug reversal is rarely a clinical issue.

Acetylcholinesterase inhibitors act to inhibit the enzyme AChE thereby preventing the enzymatic hydrolysis of ACh into choline and acetic acid. This results in an increase in ACh that is not specific to the NMJ. While nicotinic effects occur at the NMJ and autonomic ganglia, muscarinic cholinergic effects also occur at the sinus node, smooth muscle, and glands. As nondepolarizing neuromuscular agents and ACh both compete for the same postsynaptic nicotinic receptor, an increase in ACh concentration in the synaptic cleft can tip the balance of competition in favor of ACh and neuromuscular transmission is restored.

Because of an increased ACh concentration at muscarinic cholinergic receptors, bradyarrhythmias, bronchoconstriction, nausea, vomiting, diarrhea, increased intestinal peristalsis, and salivation can adversely occur. In very rare incidences cardiopulmonary arrest has been reported. For this reason, anticholinergic drugs such as atropine or glycopyrrolate should always be available to treat excessive muscarinic side effects. Several human AChE inhibitor products come premixed with an anticholinergic and clinicians should confirm what product they have before drug administration. However, in veterinary medicine it is more common practice to administer an AChE inhibitor such as edrophonium and administer an anticholinergic drug only as needed based upon side effects observed.

Edrophonium, neostigmine, and pyridostigmine differ in how they inhibit AChE activity, in their onset and duration of action, and in their incidence of muscarinic side effects. Edrophonium is a prosthetic inhibitor that produces a reversible inhibition by electrostatic attachment to the anionic site and by hydrogen bonding at the esteratic site on AChE. Neostigmine and pyridostigmine are oxydiaphoretic (acid transferring) inhibitors and inhibit AChE by forming a carbamyl–ester complex at the esteratic site of AChE to form a covalent bond. Edrophonium has the most rapid onset of action followed by neostigmine and then pyridostigmine. The muscarinic effects of edrophonium are generally mild compared to neostigmine or pyridostigmine and for this reason edrophonium is the drug most commonly selected for neuromuscular blockade reversal in veterinary medicine. The duration of action is similar for both edrophonium and neostigmine, whereas pyridostigmine is approximately 40% longer (Cronnelly et al., 1982; Morris et al., 1981). Acetylcholinesterase inhibitors undergo hepatic biotransformation and renal elimination. Patients with renal disease may have prolonged drug elimination.

Larger doses of AChE inhibitors should antagonize nondepolarizing NMB drugs more rapidly and more completely than smaller doses do. In dogs, 0.5 mg/kg IV edrophonium reversed 100% of vecuronium treated dogs while subjects receiving only 0.25 mg/kg IV required further reversal (Clutton, 1994). Neostigmine can be administered to small and large animals by slow IV injection at the dose of 0.022 mg/kg. Atropine (0.01 mg/kg large animals; 0.04 mg/kg small animals) should be administered prior to or in conjunction with neostigmine to circumvent the muscarinic effects of the latter drug (Klein et al., 1983; Jones et al., 2015).

While both metabolic and respiratory acidemia may augment the blockade induced by a nondepolarizing neuromuscular blocker, only respiratory acidemia inhibits drug reversal. Therefore, the effectiveness of AChE inhibitors may be reduced if a patient hypoventilates (Miller et al., 1974; Miller et al., 1978). Calcium channel blockers such as verapamil have been shown to potentiate nondepolarizing NMB drugs and may make achieving adequate reversal more difficult (Wali, 1986; Jones et al., 1985).

Sugammadex

Sugammadex (Org 25969) is a unique agent recently available to reverse the effects of nondepolarizing NMB agents. It is the first drug to be known as a selective relaxant binding agent (SRBA). Sugammadex is a modified γ-cyclodextran that when administered, tightly encapsulates aminosteroid-based nondepolarizing NMB agents. The cyclodextran structure has a hydrophobic cavity and a hydrophilic exterior because of the presence of polar hydroxyl groups. Hydrophobic interactions trap the NMB agent tightly in the cyclodextrin cavity and form a water-soluble guest–host complex which is readily available for elimination. This creates a concentration gradient favoring movement of the remaining nondepolarizing NMB agent away from the NMJ and its site of action and into the plasma.

Sugammadex appears to be most effective in reversal of rocuronium, followed by vecuronium, and then pancuronium. Administered at a dose of 2–8 mg/kg IV in man, the drug appears to have excellent efficacy in the face of deep neuromuscular blockade when traditional reversal with AChE inhibitors might fail. Additionally, as sugammadex does not increase the presence of ACh, it is devoid of muscarinic signs and appears to be largely free of side effects. In phase I and II trials, the most frequently reported side effects were hypotension, coughing, nausea, and xerostomia. Although cost and widespread availability have yet to make the drug's use commonplace, sugammadex has been evaluated in dogs and appears to offer promising clinical benefit. In dogs undergoing profound neuromuscular blockade with rocuronium or vecuronium, an 8 mg/kg IV dose of sugammadex results in a restoration of a TOF ratio to 0.9 in under 2 minutes with no adverse side effects.

Neostigmine or other cholinesterase inhibitors should not be used in attempts to reverse the effects of a depolarizing agent (Sunew and Hicks, 1978). Reliable chemical antidotes are not available for this group of agents. Mechanical ventilation may be required for a prolonged period. Injection of purified cholinesterase preparation has been shown to hasten recovery from the effects of succinylcholine (Scholler et al., 1977).

Because of the small therapeutic index of NMB agents, their clinical use should always be supervised by qualified experienced personnel who are thoroughly familiar with the indications, limitations, hazards, and methods of administration of these highly active drugs.

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11

Inhalation Anesthetics

Robert J. Brosnan and Eugene P. Steffey

Inhalation anesthetics are unique among the anesthetic drugs because they are administered, and in large part removed from the body, via the lungs. They are used widely for the anesthetic management of animals in part because their pharmacokinetic characteristics favor predictable and rapid adjustment of anesthetic depth. In addition, a special apparatus is usually used to deliver the inhaled agents. This helps minimize patient morbidity or mortality because it facilitates accurate and controlled anesthetic delivery, lung ventilation, and improved arterial oxygenation. Partial pressures of modern agents also rapidly equilibrate between the lung alveoli and active sites within the central nervous system (CNS). Breath-by-breath monitoring of end-tidal (alveolar) anesthetic concentrations can be routinely monitored by agent analyzers that, in effect, allow indirect breath-by-breath monitoring of drug partial pressures within CNS sites responsible for anesthetic action.

Over the nearly 170 years that inhalation anesthesia has been used in clinical practice, fewer than 20 agents have actually been introduced and approved for general use with patients. Fewer than 10 of these have had any history of widespread clinical use in veterinary medicine, and only four are currently of major clinical importance in North America (Table 11.1). All are relatively old agents, the most recent – sevoflurane – having been first synthesized and studied in the 1970s (Terrell, 2008). However, the reason for this apparent lack of drug innovation is not because the ideal inhalation anesthetic has already been identified. An ideal agent would have characteristics that include a stable shelf life without preservatives and compatibility with existing delivery equipment. It would be inexpensive to purchase, nonflammable, and easily vaporized under ambient conditions and have a high boiling point. Such an agent would have a low blood solubility to foster rapid changes in anesthetic depth and permit rapid, controlled recovery from anesthesia. The ideal agent would exhibit potency sufficient to produce surgical anesthetic planes at concentrations less than 50–60% of one atmosphere partial pressure. There would be no cardiopulmonary depression or cardiac sensitization to arrhythmias; the agent would not be irritating to airways and would have a pleasant odor (or be odorless). The drug would not lower seizure threshold, would not cause neuronal injury, and would provide analgesia (or at least not cause hyperalgesia). Finally, it would produce good skeletal muscle relaxation, resist degradation in the anesthetic delivery apparatus, undergo no biotransformation, be nontoxic to the kidneys and liver, and not cause emesis. No inhalation agent in current use fulfills all of these criteria.

Physiochemical Characteristics

The chemical structure of inhalation anesthetics and their physical properties are important to their actions and determine how these drugs are administered, what delivery and scavenge equipment is required, which techniques can be used to monitor vapor concentrations in patients, and whether or how these agents are metabolized.

Chemical Characteristics

A large number of structurally disparate agents have been delivered by inhalation to produce general anesthesia, including single atom elements (Lawrence et al., 1946a; Koblin et al., 1998), diatomic elements (Koblin et al., 1998), oxides of carbon (Thompson, 1912; Brosnan et al., 2007) and nitrogen (Wells, 1847), cyclic alkanes (Waters and Schmidt, 1934), unhalogenated (Taheri et al., 1993; Liu et al., 1994) and halogenated (Snow, 1858; Suckling, 1957; Whalen et al., 2005) n-alkanes, unhalogenated (Riggs, 1925) and halogenated (Hewer, 1942; Dingman and Lim, 1963) alkenes, unhalogenated (Jackson and Morton, 1847; Krantz et al., 1946) and halogenated (Artusio et al., 1960; Terrell, 2008) ethers, among others. With the exception of the inorganic compound nitrous oxide (N_2O), contemporary anesthetics in common use are either halogenated ethers or a halogenated alkane (Figure 11.1). Halogenation renders modern

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Table 11.1 Inhalation anesthetic agents used in animals in North America

Volatile	Gas
Major use	
Isoflurane	Nitrous oxide (N_2O)
Desflurane	
Sevoflurane	
Minor use	
Halothane	Xenon (Xe)
Enflurane	
Methoxyflurane	
Diethyl ether	

organic anesthetics nonflammable at clinically relevant concentrations.

Physical Characteristics

Clinical administration of inhalation anesthetics requires a carrier gas that must include oxygen, a source of anesthetic, and a patient breathing circuit. For very small animals (e.g., laboratory rodents or small birds) this may mean nothing more than placing the animal in a closed air-filled chamber that contains a cotton pledget saturated with liquid anesthetic (e.g., isoflurane). With larger animals and/or to provide more controlled delivery of anesthetic and O_2, it is more appropriate to use a specialized delivery apparatus to improves the safety and control of anesthetic concentrations delivered to patients. The anesthetic machine includes one or more flow meters, one or more vaporizers, and a patient breathing circuit. Reviews of vaporizers and other anesthetic delivery equipment are available elsewhere (Dorsch and Dorsch, 2007; Tranquilli et al., 2007; Miller et al., 2014).

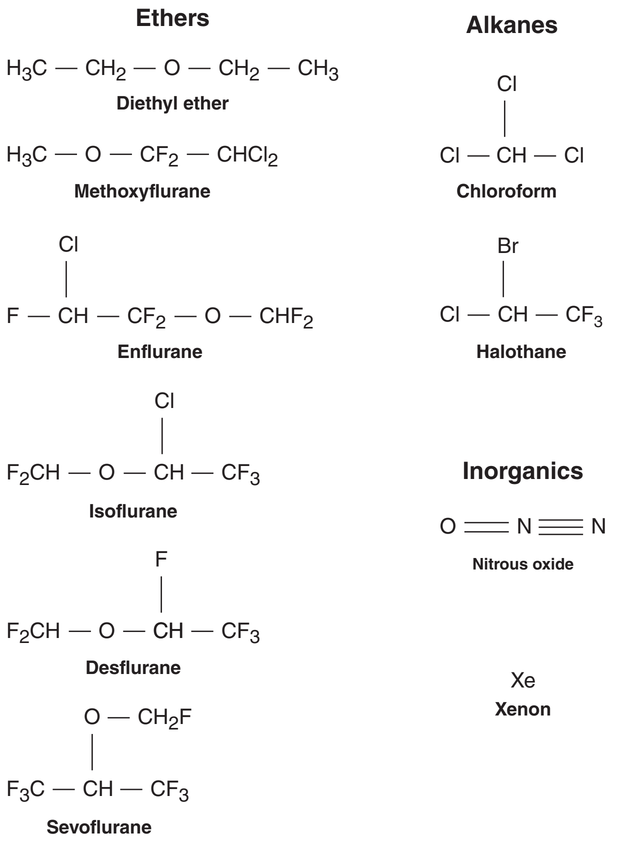


Figure 11.1 Chemical structures of inhalation anesthetics of clinical (halothane, methoxyflurane, enflurane, isoflurane, sevoflurane, desflurane), investigational (xenon), and historical (diethyl ether, chloroform) interest.

Gas versus Vapor

Inhalation anesthetics are either gases or vapors. N_2O exists as a gas under operating room conditions because at 1 atmosphere pressure, the boiling point of N_2O is −89°C. Consequently, N_2O for clinical use in tanks is compressed to the liquid state at about 750 psi (pounds per square inch; 750 psi/14.9 psi [1 atmosphere] = 50 atmospheres). As the N_2O gas is drawn from the tank, liquid N_2O is vaporized, and the overriding gas pressure remains constant until no further liquid remains in the tank. At that point, only N_2O gas remains, and the gas pressure decreases from this point as remaining gas is vented from the tank.

The term "vapor" indicates the gaseous state of a substance that at ambient temperature and pressure is a liquid; volatile anesthetics have sufficient vapor pressure to be administered via inhalation. The volatile agent desflurane has a boiling point of 23.5°C (Table 11.2); it boils (becomes a gas) only slightly above typical 20°C ambient room temperature or at altitudes higher than 1100 meters (3600 feet) due to decreased barometric pressure. As a result, a specialized vaporizer is required to maintain temperature and pressure constant and allow for precise and safe delivery of this agent.

Methods of Description

Quantities of inhalation anesthetic agent are usually characterized by one of three methods: pressure (e.g., in mmHg), concentration (in volumes %), or mass (in mg or g). The form most familiar to clinicians is that of concentration (e.g., *X*% of agent A in relation to the whole gas mixture). Modern monitoring equipment samples inspired and expired gases and provides concentration readings for inhalation anesthetics. Precision vaporizers used to control delivery of inhalation anesthetics are calibrated in percentage of agent, and effective doses are almost always reported in percentages. Finally, the molecular weight and agent density are used in ideal gas calculations to convert from liquid to vapor volumes and mass (Hill, 1980).

Table 11.2 Some physiochemical properties of volatile anesthetics. Source: Data from Lerman et al., 1983; Taheri et al., 1991; Soares et al., 2012.

Property	Desflurane	Halothane	Isoflurane	Sevoflurane	Enflurane	Methoxyflurane
Molecular weight	168	197	185	200	185	165
Liquid specific gravity (20°C) (g/ml)	1.47	1.86	1.49	1.52	1.52	1.42
Boiling point(°C)	23.5	50	49	59	57	105
Vapor pressure						
20°C (68°F)	664	244	240	160	172	23
24°C (75°F)	798	288	286	188	207	28
ml of vapor/mL of liquid at 20°C	209.7	227	195	183	198	207
Partition coefficients						
saline/gas (37°C)	0.287	0.825	0.517	0.329	0.713	4.0
olive oil/gas (37°C)	19.2	224	98.9	51.3	103	611

Vapor Pressure

The vapor pressure of an anesthetic is an indicator of its ability to evaporate; it reflects the tendency for molecules in the liquid state to enter the gaseous (vapor) form. The vapor pressure of a potent volatile anesthetic must be sufficient to provide enough molecules of anesthetic in the vapor state to produce anesthesia at ambient conditions. The *saturated vapor pressure* represents a maximum concentration of molecules in the vapor state that can exist for a given liquid at each temperature. The *saturated vapor concentration* can be easily determined by relating the vapor pressure to the ambient pressure. Using halothane and associated information from Table 11.2 as an example, we see that a maximal concentration of 32% halothane is possible under usual operating-room conditions; that is, $244/760 \times 100 = 32\%$, where 244 mmHg is the vapor pressure at 20°C and 760 mmHg is the barometric pressure at sea level. Thus, other variables considered constant, the greater the vapor pressure, the greater the concentration of the drug deliverable to the patient.

Properties Influencing Drug Kinetics: Solubility

As described by Henry's Law, the partial pressure exerted by a concentration of gas in solution is inversely proportional to the solubility of that gas in that solvent. The solubility of an anesthetic is a major characteristic of the agent and has important clinical ramifications. For example, anesthetic solubility in blood and body tissues is a primary factor in the rate of uptake and distribution within the body. It is therefore a primary determinant of the speed of anesthetic induction and recovery. Solubility in lipid bears a strong relationship to anesthetic potency, and the tendency to dissolve in anesthetic delivery components such as rubber influences equipment selection and other aspects of anesthetic management.

The extent to which a gas will dissolve in a given solvent is usually expressed in terms of its *partition coefficient* (PC)(Table 11.3), a distribution constant that describes the concentration ratio of a gas between two bulk phases (e.g., between blood and gas) at a specified temperature. It thus describes the affinity or capacity or solubility of an anesthetic for one solvent phase relative to another, that is, how the anesthetic will *partition* itself between two phases after equilibrium has been reached. Anesthetic gas movement occurs because of a partial pressure difference in the two phases so that when there is no longer any anesthetic partial pressure difference between the two phases, there is no longer any net movement of anesthetic in either phase direction, and equilibrium has been achieved. Solvent/gas and human tissue/gas PCs are summarized in Table 11.3; some of these differ significantly from values in animals for some agents (Soares et al., 2012).

Table 11.3 Partition coefficients at 37°C. Source: Data from Eger, 1981; Goto et al., 1998; Lynch et al., 2000; Eger et al., 2003a.

Agent	Blood/gas	Oil/gas	Brain/blood	Heart/blood	Liver/blood	Kidney/blood	Muscle/blood	Fat/blood
Desflurane	0.45	19	1.2	1.2	1.5	0.9	1.7	29
N_2O	0.46	1.4	1.1	1.0	–	–	1.2	2.4
Sevoflurane	0.65	47	1.7	1.7	2.0	1.2	2.6	52
Isoflurane	1.4	98	1.6	1.6	1.9	1.0	2.6	50
Enflurane	2.0	96	1.4	–	1.9	1.0	1.1	42
Halothane	2.4	224	1.9	1.7	2.3	1.3	2.9	57
Methoxyflurane	15	970	1.3	–	1.9	0.7	1.0	60
Xenon	0.12	1.8	1.6	–	–	0.9	0.9	–

Of all the PCs that have been described or are of possible interest, two are of particular importance in the practical understanding of anesthetic management. They are the blood/gas and the oil/gas partition coefficients.

Blood/Gas Partition Coefficient

The blood/gas solubility (Table 11.3) is a determinant of the speed of anesthetic onset (induction or change in anesthetic level) and offset (recovery). All else being equal, a lower blood/gas PC allows for more rapid anesthetic induction or rate of change of anesthetic level in response to a stepwise change in anesthetic delivery.

Oil/Gas Partition Coefficient

The oil/gas PC is another solubility characteristic of clinical importance (Table 11.3). This PC describes the ratio of concentration of an anesthetic in oil (in this case olive oil is the generally agreed upon standard) and gas phases at equilibrium. The oil/gas PC correlates inversely with anesthetic potency and describes the capacity of lipids for anesthetic.

Other Partition Coefficients

Solubility characteristics for tissues (Table 11.3) and other media like rubber and plastic (components of anesthetic delivery equipment; Table 11.4) are also important. For example, tissue solubility determines in part the quantity of anesthetic removed from the blood to which it is exposed. The higher the tissue solubility, the more anesthetic is removed from the blood, which slows the rate of rise of anesthetic partial pressure at active sites. During recovery, anesthetic can leave these tissue reservoirs and oppose anesthetic washout and delay recovery time. High agent solubility in rubber (Table 11.4) will oppose the rise of anesthetic partial pressure within the anesthetic apparatus (such as in an equine anesthetic circuit that often uses a substantial quantity of rubber in hoses and breathing bags) and slow anesthetic delivery to the patient.

Table 11.4 Rubber or plastic/gas partition coefficients at room temperature. Source: Data from Marx et al., 1996; Eger et al., 2003a.

Agent	Latex Rubber	Silicone Rubber	Polyvinyl chloride	Polyethylene
Desflurane	19	–	35	16
N_2O	1.2	–	–	–
Sevoflurane	29	–	68	31
Isoflurane	49	67	110	2
Enflurane	74	–	120	2
Halothane	190	165	190	26
Methoxyflurane	630	–	–	118

Pharmacokinetics: Uptake and Elimination of Inhalation Anesthetics

The aim in administering an inhalation anesthetic to a patient is to achieve partial pressure or tension of anesthetic (P_{anes}) in the CNS to achieve a desired level of anesthesia. Movement of molecules of inhalation anesthetics, like O_2 and CO_2, occurs down partial pressure gradients (Figure 11.2). Gases move from regions of higher tension to those of lower tension until equilibrium (i.e., equal partial pressure in the two media) is established. Anesthetic partial pressure is highest immediately downstream from the vaporizer and is progressively less as gas travels to a semiclosed anesthetic breathing circuit, from circuit to lungs, from lungs to arterial blood, and, finally, from arterial blood to body tissues such as the brain and spinal cord (Figure 11.2). With modern drugs, the anesthetic partial pressure in the pulmonary alveoli rapidly equilibrates the anesthetic partial pressure in highly vascular tissues receiving large arterial blood flow (such as sites of anesthetic action within the CNS). Hence, the alveolar partial pressure (P_A) of anesthetic is in a delayed

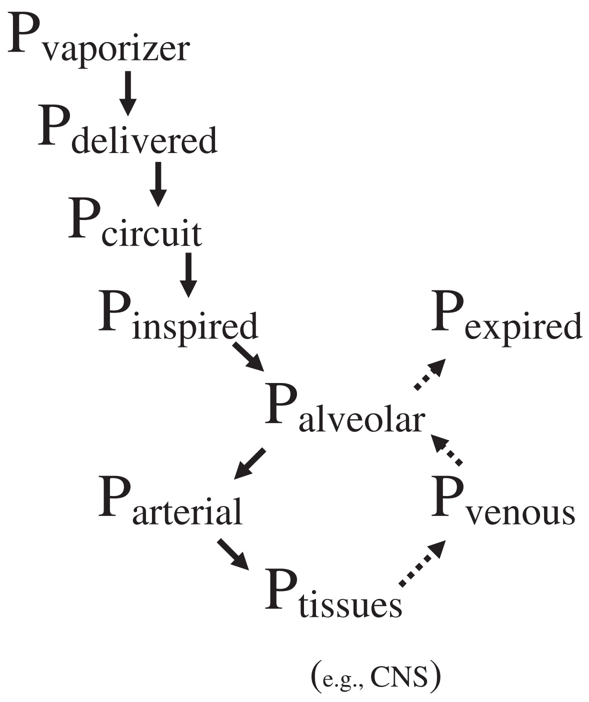


Figure 11.2 The movement of inhaled anesthetic agents down a series of partial pressure gradients (gas tension gradients) during induction and recovery. During induction there is a high anesthetic tension in the vaporizer that decreases progressively towards the site of action in the CNS.

Table 11.5 Factors that promote a rapid change in alveolar anesthetic tension

- A. Increased alveolar delivery of anesthetic
  - 1. Increased inspired anesthetic concentration
    - a. Increased vaporization of agent
    - b. Increased vaporizer dial setting
    - c. Increased anesthetic-laden fresh gas inflow to the patient breathing circuit
    - d. Decreased gas volume of patient breathing circuit
    - e. Vaporizer positioned in a patient rebreathing circuit
  - 2. Increased alveolar ventilation
    - a. Increased minute ventilation
    - b. Decreased ventilation of respiratory system dead space
- B. Decreased removal of anesthetic from the alveoli
  - 1. Decreased blood solubility of anesthetic
  - 2. Decreased cardiac output
  - 3. Decreased alveolar–venous anesthetic gradient

equilibrium with the CNS partial pressure of anesthetic. Monitoring drug alveolar partial pressure allows indirect real-time monitoring of the effect site concentration.

The P_A of anesthetic is a balance between anesthetic input (i.e., delivery to the alveoli) and loss (uptake by blood and body tissues) from the lungs (Figure 11.2). A rapid rise in P_A of anesthetic is associated with a rapid anesthetic induction or change in anesthetic depth. Factors that contribute to a rapid change in P_A of anesthetic are summarized in Table 11.5.

Delivery to the Alveoli

Delivery of anesthetic to the alveoli – and the rate of rise of the alveolar concentration or fraction (F_A) toward the inspired concentration or fraction (F_I) – depends on the inspired anesthetic concentration itself and the magnitude of alveolar ventilation. Increasing either one of these or both increases the rate of rise of the P_A of anesthetic.

Inspired Concentration

The upper limit of inspired concentration is dictated by the agent vapor pressure at a given temperature. Characteristics of the patient breathing system are also important, including the type of circuit (open, semiopen, semiclosed, or closed), the amount of rubber or plastic in the system, the position of the vaporizer relative to the breathing circuit (i.e., within or outside the circuit), and the fresh gas inflow to the patient breathing circuit (Eger, 1974; Dorsch and Dorsch, 2007; Tranquilli et al., 2007; Brosnan, 2013; Miller et al., 2014).

Alveolar Ventilation

An increase in alveolar ventilation increases the rate of delivery of inhalation anesthetic to the alveoli. If unopposed, alveolar ventilation would rapidly increase the alveolar concentration of anesthetic so that within minutes the alveolar concentration would equal the inspired concentration. However, in reality, the input created by alveolar ventilation is initially diluted by the volume of the lung's functional residual capacity (FRC) and subsequently by absorption of anesthetic into blood. Hypoventilation reduces the rate at which the alveolar concentration increases over time compared to the inspired concentration.

Removal from Alveoli: Uptake by Blood

Anesthetic uptake is the product of three factors: solubility (S; the blood/gas solubility, Table 11.3), cardiac output (CO), and the difference in the anesthetic partial pressure between the alveoli and venous blood returning to the lungs ($P_A{-}P_v$; expressed in mmHg); that is,

$$\text{Uptake} = \text{S} \times \text{CO} \times [(P_A - P_v)/P_{bar}]$$

where P_{bar} = barometric pressure in mmHg (Eger, 1974). Note that if any of these three factors equals zero, there is no further uptake of anesthetic by blood.

Solubility

The solubility of an inhalation anesthetic in blood and tissues is characterized by its partition coefficient (PC; Table 11.3). Compared to an anesthetic agent with high blood solubility (PC), an agent with low blood solubility should be associated with a more rapid equilibration between tissue phases because a large amount of the highly soluble anesthetic must be dissolved in the blood before equilibrium is reached with the gas phase. In the case of the agent with a high blood/gas PC, the blood acts like a large "sink" into which the anesthetic is poured, and thus a large blood anesthetic concentration is required before a large blood anesthetic partial pressure can be reached. It is the partial pressure gradient, and not the concentration gradient, that determines diffusion of gases. As a result, it takes more soluble anesthetics more time to reach a given blood partial pressure and CNS partial pressure than less soluble anesthetics. Anesthetic agents with a low blood/gas PC are desirable because they permit: (i) a more rapid anesthetic induction (i.e., more rapid rate of rise in alveolar concentration during induction; Figure 11.3); (ii) more precise control of anesthetic depth (i.e., alveolar concentration during the anesthetic maintenance phase of anesthesia); and (iii) a more rapid elimination of anesthetic and recovery from anesthesia (i.e., a rapid decrease in alveolar concentration during the anesthetic recovery phase).

Cardiac Output

Cardiac output, the amount of blood flowing through the lungs (or systemic circulation) per unit time, influences anesthetic uptake from the lungs. With greater cardiac output, there is a greater quantity of blood passing through the lungs that will take up anesthetic from the

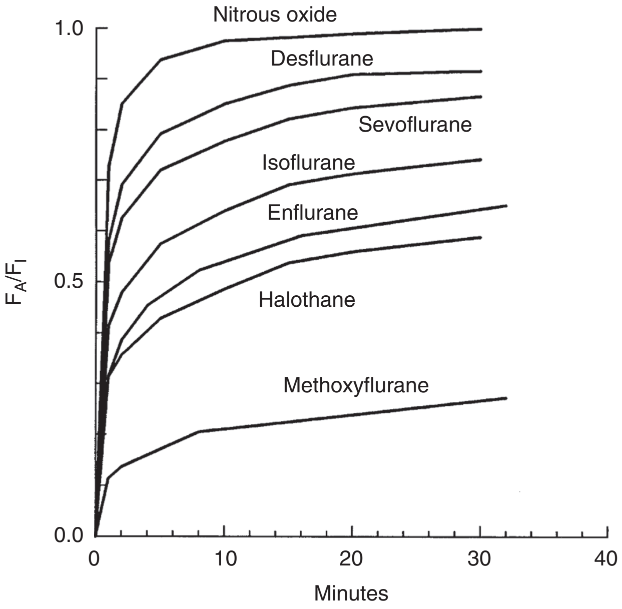


Figure 11.3 The rise in the alveolar (F_A) anesthetic concentration toward the inspired (F_I) concentration in humans. Note the rise is most rapid with the least soluble anesthetic, N_2O, and slowest with the most soluble anesthetic, methoxyflurane. Source: Data from Eger and Eger, 1985; Eger, 1992.

alveoli. A large CO, like increased anesthetic agent blood solubility, delays the alveolar rise of P_{anes} and anesthetic onset.

Alveolar to Venous Anesthetic Partial Pressure Difference

The magnitude of difference in anesthetic partial pressure between the alveoli and venous blood is related to the amount of uptake of anesthetic agent by tissues. Not surprisingly, the largest gradient occurs during induction. Once the tissues no longer absorb anesthetic (i.e., equilibrium between the two phases is reached), there is no longer any uptake of anesthetic agent from the lungs; the venous blood returning to the lungs contains as much agent as when it left the lungs. The changes in gradient in between these extremes result from the relative distribution of CO. In this regard it is important to recognize that roughly 70–80% of the CO is normally directed to only a small volume of body tissues in a lean individual (Table 11.6) (Eger, 1974; Staddon et al., 1979; Yasuda et al., 1990; Eger and Saidman, 2005). Tissues such as brain, heart, hepatoportal system, and kidneys represent only about 10% of the body mass but normally receive about 75% of the total blood flow each minute. These highly perfused tissues equilibrate relatively rapidly with the arterial anesthetic tension. Since the venous anesthetic tension equals that in the tissue within 10–15 minutes, about 75% of the blood returning to the lungs is the same as the alveolar tension, presuming that arterial anesthetic tension is held constant. Thus anesthetic uptake is reduced over time. Skin and muscle compose the major bulk of the body (about 50% in humans) but at rest receive only about 15–20% of the CO, so saturation of this tissue group may require up to a few hours to accomplish. Fat is a variable component of body bulk and receives only a small proportion of blood flow. Consequently, anesthetic saturation of this tissue group is very slow, especially given that all anesthetics are considerably more soluble in fat than in other tissue groups (Table 11.3).

Table 11.6 Blood flow characteristics in different tissue groups. Source: Eger, 1974. Reproduced with permission of El Eger.

	Tissue group[a]			
	Vessel rich	Muscle	Fat	Vessel poor
% of body mass	10	50	20	20
% of cardiac output	75	19	5	<1

[a] Brain, heart, hepatoportal, kidney are examples of components of the "vessel rich" grouping and ligaments, tendons, bone are examples within the "vessel poor" tissue grouping.

Other factors can further influence the magnitude of the alveolar-to-arterial anesthetic partial pressure gradient. They include body size (Wahrenbrock et al., 1974), abnormalities of lung ventilation/perfusion (Eger and Severinghaus, 1964), loss of anesthetic via the skin (Stoelting and Eger, 1969b; Fassoulaki et al., 1991; Lockhart et al., 1991b) and into closed gas spaces (Eger, 1974; Eger, 1985; Eger et al., 2003a), and metabolism (Eger, 1974; Eger et al., 2002; Eger et al., 2003a).

Anesthetic Recovery

Recovery from inhalation anesthesia is a consequence of anesthetic elimination from the CNS. This first requires washout of anesthetic from the alveoli and blood (Figure 11.2). Factors favoring a faster rate of rise of CNS anesthetic partial pressure are the same factors that favor faster anesthetic elimination: alveolar ventilation, CO, and the agent blood/gas partition coefficient. Indeed, graphs representing anesthetic wash-out from the alveoli versus time (Figure 11.4) are essentially inverse of the anesthetic wash-in curves (Figure 11.3).

Anesthetic elimination kinetics also exhibit context sensitivity with respect to time; drug washout is slower as the duration of anesthesia increases. The relative context sensitivity increases as a function of the agent solubility in blood and body tissues (Eger and Johnson, 1987; Eger, 1993; Eger et al., 1998; Eger and Saidman, 2005; Eger and Shafer, 2005). During anesthetic maintenance, agent concentrations gradually increase in less perfused muscle and viscera and, more slowly, in fat. These large tissue volumes become an ever increasing reservoir of anesthetic until their partial pressure equilibrates with that

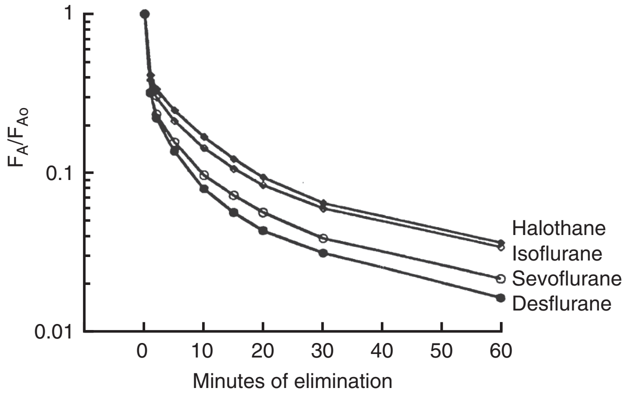


Figure 11.4 The fall in alveolar (F_A) concentration relative to the alveolar concentration at the end of anesthesia (F_{A0}). Note that the newest, most insoluble volatile anesthetic, desflurane, is eliminated in humans more rapidly than the other contemporary potent anesthetics. Not shown is information for methoxyflurane; if present the curve for methoxyflurane would appear above that for halothane. Source: Eger, 1992. Reproduced with permission of Wolters Kluwer Health.

of the arterial blood. During recovery, gradual anesthetic washout from less vascular tissues can oppose the fall in anesthetic partial pressure in the blood and redistribute to the CNS, thereby delaying anesthetic recovery.

Other factors contributing to anesthetic elimination from the body include percutaneous loss (Stoelting and Eger, 1969b; Cullen and Eger, 1972; Fassoulaki et al., 1991; Lockhart et al., 1991b), intratissue (Neumann et al., 2005) or intertissue (Carpenter et al., 1986b; Carpenter et al., 1987) diffusion of agents, and body size and composition (Lemmens et al., 2008). Metabolism plays a small role with some older inhalation anesthetic agents, such as methoxyflurane or halothane (Cahalan et al., 1981; Carpenter et al., 1986a; Carpenter et al., 1987), but not with newer volatile agents which are largely eliminated unchanged by the respiratory system.

Anesthetic Dose: The Minimum Alveolar Concentration

The standard index of potency for inhalation anesthetics is the minimum alveolar concentration (MAC) (Merkel and Eger, 1963; Eger et al., 1965; Eger, 2002), the alveolar concentration of an inhaled anesthetic that prevents gross nonreflexive movement in 50% of subjects exposed to a supramaximal noxious stimulus. Thus, MAC is equivalent to the median effective dose, or ED_{50}, for inhaled anesthetics. The dose that corresponds to the ED_{95} (95% of the individuals are anesthetized), at least in humans, is 20–40% greater than MAC (de Jong and Eger, 1975); and 2.0 times the MAC (i.e., 2 MAC) represents a deep level of anesthesia, in some cases even an anesthetic overdose. MAC values for contemporary inhalation anesthetics in select animal species are summarized in Table 11.7.

Table 11.7 The minimum alveolar concentration (MAC) of inhalation anesthetics. Source: Data from Lynch et al., 2000; Steffey and Mama, 2007.

Anesthetic	Cat	Dog	Horse	Human
Methoxyflurane	0.23	0.29	0.28	0.16
Halothane	1.04	0.87	0.88	0.74
Isoflurane	1.63	1.30	1.31	1.15
Enflurane	2.37	2.06	2.12	1.68
Sevoflurane	2.58	2.36	2.31	2.05
Desflurane	9.79	7.20	8.06	7.25
Xenon	–	119	–	71
Nitrous oxide	255	222	205	104

The anesthetic potency of an inhaled anesthetic is inversely related to MAC (i.e., potency = 1/MAC). MAC of conventional anesthetics is inversely related to the oil/gas PC, as described by the Meyer–Overton relationship (Figure 11.5) (Meyer, 1899; Overton, 1991). Thus, a very potent anesthetic (e.g., methoxyflurane) has a low MAC value and a high oil/gas PC; an agent of low anesthetic potency (e.g., N_2O) has a high MAC and a low oil/gas PC.

It is important to note that MAC is normally determined in healthy animals under controlled laboratory conditions in the *absence* of other drugs and other circumstances common to clinical use that may modify anesthetic requirements. Many factors may either increase or decrease MAC (anesthetic requirement). The interested reader is referred elsewhere for reviews of this topic (Stoelting and Hillier, 2006; Tranquilli et al., 2007; Miller et al., 2014).

Pharmacodynamics: Actions and Toxicity of the Inhalation Anesthetics

By virtue of their effects on multiple cellular targets, inhaled anesthetics have widely distributed and largely depressant effects on function of most body systems. Though the magnitude of some actions vary with agent, the effect profile of most potent modern alkane and ether type anesthetics is remarkably similar. In contrast, the less potent inorganic anesthetics (N_2O and Xe) may not produce similar anesthetic endpoints and side effects in all species at standard pressures. Following is an overview of major inhalation anesthetic effects on body systems with a focus on drug effects in healthy patients. However, the reader is cautioned that effects both desirable (e.g., immobilization potency) and adverse (e.g., cardiorespiratory depression) may be modified by a number of variables encountered in clinical and laboratory settings,

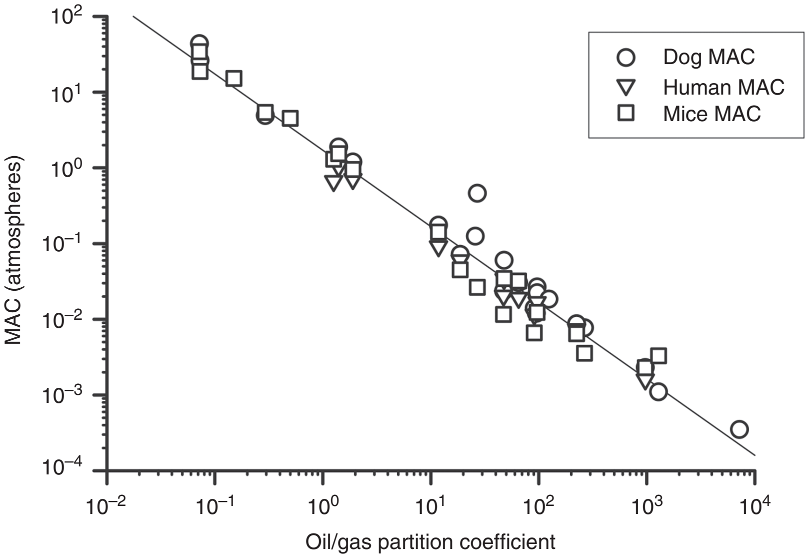


Figure 11.5 Oil/gas partition coefficients versus potency (MAC) of inhalation anesthetics in dogs, humans and mice. Source: Miller et al., 2014. Reproduced with permission of Elsevier.

including species, genetics, duration of anesthesia, noxious (surgical) stimulation, ventilation mode, coexisting disease, and concurrent medications. We refer the reader to additional references that discuss these interactions in greater detail (Tranquilli et al., 2007; Miller et al., 2014).

Central Nervous System

Inhalation anesthetics produce a reversible generalized CNS depression for which the degree of depression is often described as depth of anesthesia. The components of general anesthesia have traditionally been considered to include: unconsciousness, amnesia, analgesia, and immobility. Some also include in this concept, suppression of autonomic reflexes. Most recently, in attempting to better define the site(s) and mechanism(s) of action of inhalation anesthetics this list has been distilled to just two reversible qualities applying to all inhalation agents: amnesia and immobility in response to a noxious stimulus (Eger et al., 2003a; Eger and Sonner, 2006).

The inhaled agents cause immobility and amnesia, but these distinct endpoints are achieved via actions at different CNS sites. In an experimental goat model in which the cerebral circulation was isolated from the rest of the body, selective brain isoflurane administration more than doubled the anesthetic requirement for immobility compared to whole-body isoflurane administration. This demonstrated that the spinal cord, and not the brain, is principally responsible for preventing movement during surgery with inhaled anesthetics (Antognini and Schwartz, 1993). Within the spinal cord, immobility is most likely produced by depression of locomotor neuronal networks located in the ventral horn (Jinks et al., 2005).

In contrast, amnestic effects of inhaled anesthetics are produced by actions within the brain, most probably within the amygdala and hippocampus (Eger et al., 2003b). Lesions created within the amygdala of rats can block amnestic actions of sevoflurane (Alkire and Nathan, 2005). On electroencephalography, hippocampal-dependent θ-rhythm frequency slows in proportion to the amnestic effects observed with subanesthetic concentrations of isoflurane in rats (Perouansky et al., 2010). Furthermore, mutant mice lacking the gene encoding either the α_4 or β_3 subunits of $GABA_A$ receptors are resistant to isoflurane depression of hippocampus-dependent learning and memory (Rau et al., 2009; Rau et al., 2011), and antagonism of α_5 subunit-containing $GABA_A$ receptors restores hippocampal-dependent memory during sevoflurane administration (Zurek et al., 2012). Clearly the mechanisms of inhaled anesthetic action responsible for amnesia are different from those responsible for immobility.

At concentrations above MAC, it is presumed that inhaled anesthetics sufficiently depress cortical function to prevent animals from experiencing motivational-affective dimensions of pain (Melzack and Casey, 1967). Additionally, concentrations of modern volatile agents (halothane, enflurane, isoflurane, sevoflurane, desflurane) between 0.8 and 1.0 times MAC decrease (but do not ablate) wind-up and central sensitization and so may help prevent heightened postoperative pain sensitivity; however, higher concentrations of contemporary drugs offer no further benefit in this regard (O'Connor and Abram, 1995; Mitsuyo et al., 2006). Volatile anesthetic concentrations between 0.4 and 0.8 times MAC decrease withdrawal responses to noxious stimuli, but lower concentrations can actually cause hyperalgesia with a peak effect at 0.1 times MAC (Sonner et al., 1998; Zhang et al., 2000) due to potent nicotinic cholinergic receptor inhibition (Flood et al., 2002). In contrast, the gaseous anesthetics xenon and nitrous oxide produce analgesia via glutamatergic receptor inhibition (Yamakura and Harris, 2000), and in the case of nitrous oxide, through additional modulation of noradrenergic–opioid receptor pathways (Zhang et al., 1999). Anatomically, analgesic actions occur both supraspinally and within the dorsal horn of the spinal cord, and are likely responsible for greater blunting of autonomic responses associated with these gases as compared to the modern volatile agents (Nakata et al., 1999).

Unconsciousness, at least insofar as it may be correlated with loss of the righting reflex and spontaneous movement (Quasha et al., 1980), may occur at yet other sites within the CNS. Nicotine injections into the central medial thalamus causes partial-to-full arousal in rats lightly anesthetized with sevoflurane, suggesting a role of nicotinic acetylcholine receptors within this region (Alkire et al., 2007). Within the midbrain, electrical stimulation (Solt et al., 2014) or injection of D_1 receptor agonists (Taylor et al., 2013) into the ventral tegmental area reverses the immobilizing effects of isoflurane in rats, thus indicating a role for dopamine receptors. Presumably, thalamic and brainstem arousal allows subsequent activation of other cortical regions responsible for information processing and integration, a hallmark of consciousness (Tononi, 2008).

Of the contemporary inhaled anesthetics, enflurane (Joas et al., 1971; Neigh et al., 1971) and sevoflurane (Adachi et al., 1992; Komatsu et al., 1994; Osawa et al., 1994; Woodforth et al., 1997; Hilty and Drummond, 2000) are capable of producing epileptiform activity during electroencephalography. However, tonic–clonic muscle activity is typically only observed with enflurane, whereas sevoflurane may actually raise the threshold for seizure manifestation (Karasawa, 1991; Fukuda et al., 1996; Murao et al., 2000a), although perhaps less so than other inhaled agents (Murao et al., 2002). Isoflurane (Koblin et al., 1981; Fukuda et al., 1996; Murao et al., 2000a, 2000b) and desflurane (Fang et al., 1997b) suppress convulsive activity induced by such drugs as bupivacaine, lidocaine, and penicillin.

Inhalation anesthetics are cerebral vasodilators and tend to increase cerebral blood flow and interfere with normal cerebral vascular autoregulation. These effects are agent dependent (less with sevoflurane) (Conzen et al., 1992; Gupta et al., 1997; Matta et al., 1999; Nishiyama et al., 1999; Summors et al., 1999; Bedforth et al., 2000), dose dependent (less at sub-MAC concentrations) (Bundgaard et al., 1998; Matta et al., 1999; Werner et al., 2005), and species dependent (autoregulation better preserved in horses than humans and small animals) (Brosnan et al., 2011). Cerebrovasodilation increases intracranial blood volume and pressure, resulting in increased intracranial pressure that is exacerbated by head-down body positioning (Brosnan et al., 2002a, 2002b), hypercapnia (Brosnan et al., 2003a, 2003b), prolonged anesthetic duration (Brosnan et al., 2003a), and pathophysiological changes associated with increased intracranial elastance (Brosnan et al., 2004), such as intracranial masses, hemorrhage, edema, and hydrocephalus (North and Reilly, 1990). Severe and uncompensated increases in intracranial pressure can cause cerebral herniation and also decrease the extracranial-to-intracranial pressure gradient, defined as the cerebral perfusion pressure, resulting in cerebral ischemia.

In the adult, inhaled anesthetic preconditioning offers some degree of neuroprotection during subsequent hypoxic or ischemic insults (Xiong et al., 2003; Kehl et al., 2004; Zheng and Zuo, 2004; Payne et al., 2005; Li et al., 2008; Li and Zuo, 2009; Yang et al., 2011). However, in neonates, many inhaled agents can adversely affect neural viability or neural development. Contemporary volatile anesthetics have been associated with neuronal apoptosis in the brains and spinal cords of neonatal rats (Sanders et al., 2008b; Istaphanous et al., 2011) and monkeys (Brambrink et al., 2010, 2012); the longer-term clinical consequences for behavioral changes or learning and memory deficits are presently unresolved (Jevtovic-Todorovic et al., 2003b; Culley et al., 2004; Loepke et al., 2009; Satomoto et al., 2009; Kodama et al., 2011). In the aged, inhaled agents have been associated with postanesthetic cognitive dysfunction (Culley et al., 2003; Zhang et al., 2012) with the suggestion that impairment could be secondary to accumulation of amyloid protein within the brain (Xie et al., 2006). Although important in animals, implications of these potential side effects are even greater for humans.

Respiratory System

All contemporary inhalation anesthetics depress alveolar ventilation. As a consequence, the P_aCO_2 is increased in direct relation to anesthetic dose (MAC multiple; Figure 11.6). The magnitude of P_aCO_2 is also species related (Figure 11.7). In addition, the normal stimulation to ventilation caused by an increased P_aCO_2 and/or a low arterial oxygen tension (P_aO_2) is reduced (Knill and Gelb, 1978; Knill et al., 1983; Dahan et al., 1994, 1996; Sarton et al., 1996; Sjogren et al., 1998, 1999; Miller et al., 2014).

Bronchospasm is associated with some diseases and other patient conditions and contributes to increased airway resistance. Among the earlier available volatile anesthetics, halothane was the most effective bronchodilator (Klide and Aviado, 1967; Coon and Kampine, 1975). Subsequent work with isoflurane, sevoflurane, and desflurane indicates the relaxation of constricted bronchial muscles by these agents is equal to or exceeds that caused by halothane (Hirshman and Bergman, 1978; Mazzeo et al., 1996; Rooke et al., 1997; Habre et al., 2001; Dikmen et al., 2003), although very deep anesthetic planes (≥2

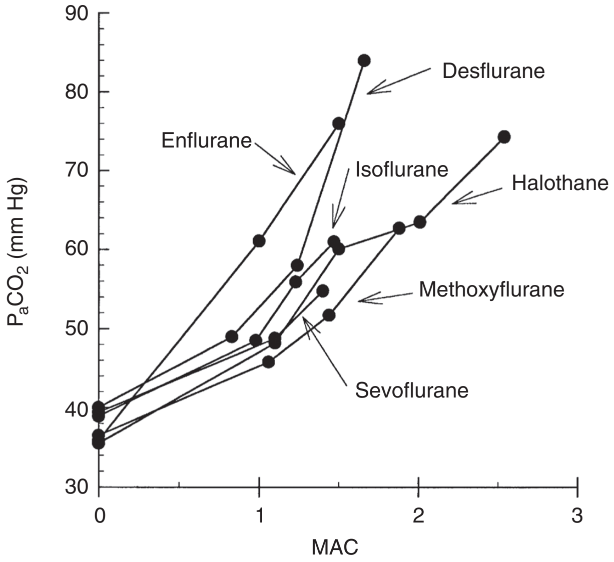


Figure 11.6 A summary of the effects of contemporary volatile anesthetics on P_aCO_2 in humans. Source: Data from Munson et al., 1966; Larson et al., 1969; Calverley et al., 1978; Doi and Ikeda, 1987a; Lockhart et al., 1991a.

times MAC) of desflurane may be associated with loss of bronchodilation, possibly due to release of tachykinin (Dikmen et al., 2003; Nyktari et al., 2006; Satoh et al., 2009).

Volatile inhalation anesthetics may inhibit reflex hypoxic pulmonary vasoconstriction and thereby contribute to a maldistribution of ventilation to perfusion and an increase in the alveolar-to-arterial partial pressure of oxygen and decrease in P_aO_2 (Marshall et al., 1984; Miller et al., 2014). However, results of in vivo studies of clinically relevant concentrations of volatile anesthetics suggest minimal or no inhibitory effects (Mathers et al., 1977; Fargas-Babjak and Forrest, 1979; Domino et al., 1986; Lesitsky et al., 1998; Kerbaul et al., 2000, 2001).

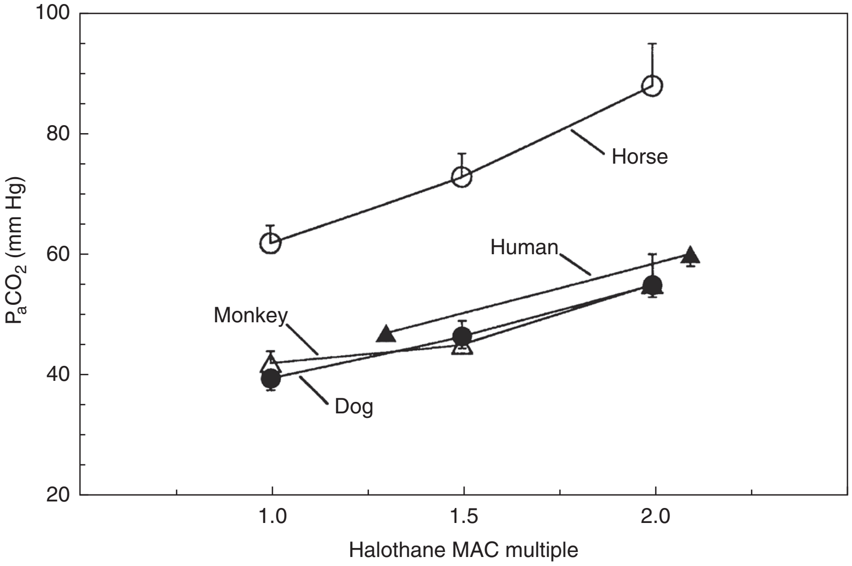
Airway irritation caused by inspiring an inhalation anesthetic is undesirable. Airway irritation has not been generally recognized as a problem associated with induction of anesthesia in animals. However, human patients commonly react by breath holding, coughing, and laryngospasm when exposed to concentrations of 7% or more desflurane early in the course of anesthesia (Eger, 1993; TerRiet et al., 2000). As a result, desflurane is not commonly used for anesthetic induction in human patients.

Cardiovascular System

Inhalation anesthetics may produce drug specific and/or dose-related effect. Such effects may manifest as changes in arterial blood pressure, cardiac output, stroke volume, heart rate, and/or rhythm of heart beat. Such changes may be caused by effects on myocardial contractility, peripheral vascular smooth muscle, and/or autonomic nervous system tone. Effects imposed by inhalation anesthetics may be further impacted by controlled versus spontaneous ventilation, concurrently administered drugs with direct or indirect hemodynamic actions, and preexisting cardiovascular disease.

All of the volatile anesthetics decrease cardiac output, usually via a decrease in myocardial contractility (Eger, 1981; Eisele, 1985; Pagel et al., 1991b; Boban et al., 1992; Warltier and Pagel, 1992; Pagel et al., 1993) and, in turn, stroke volume. The magnitude of change is dose related and dependent on the agent (Klide, 1976; Steffey and Howland, 1978a, 1980; Eger, 1981; Merin et al., 1991; Warltier and Pagel, 1992; Mutoh et al., 1997; Hanouz et al., 2000). Arterial blood pressure is also decreased in a dose-related manner (Figure 11.8) (Steffey et al., 1974b,

Figure 11.7 The P_aCO_2 (mean ± SE; mm Hg) in spontaneously breathing, health dogs, horses, humans and monkeys during halothane-oxygen anesthesia. The anesthetic dose is expressed as a multiple of the minimum alveolar concentration (MAC) for each species. Source: Steffey, 2009.



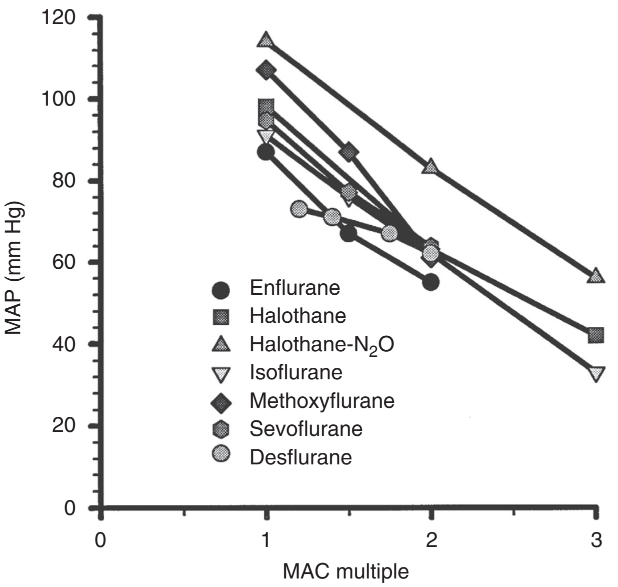


Figure 11.8 Inhalation anesthetics dose-dependently (expressed as multiples of MAC) decrease mean arterial blood pressure (MAP) in mechanically-ventilated normocapnic dogs. Source: Data from Steffey et al., 1975; Steffey and Howland, 1978a; Steffey et al., 1984; Merin et al., 1991; Frink et al., 1992b.

1974c; Steffey and Howland, 1977; Merin et al., 1991; Frink et al., 1992b; Mutoh et al., 1997). The decrease in arterial blood pressure is usually related to a decrease in cardiac output and a decrease in peripheral vascular resistance, the relative contributions of which varies between species.

All volatile anesthetics depress myocardial contractility via alterations of intracellular calcium homeostasis at several subcellular locations in normal cardiac muscle (Bosnjak et al., 1991; Davies et al., 2000; Huneke et al., 2001; Park et al., 2007). The rank order of depression of contractile function is halothane = enflurane > isoflurane = desflurane = sevoflurane (Miller et al., 2014). Volatile anesthetics can provide important beneficial effects on mechanical function during myocardial ischemia and myocardial reperfusion injury. For a more complete review of mechanisms of action of volatile anesthetics on myocardial function readers are referred to the recent review by Pagel and colleagues (Miller et al., 2014).

Inhalation anesthesia may alter the normal distribution of blood flow to organs. Specific effects are agent, dose, and time related (Vatner and Smith, 1974; Manohar and Parks, 1984a, 1984b; Manohar and Goetz, 1985; Bernard et al., 1991; Merin et al., 1991; Hartman et al., 1992; Eger et al., 2003a). For example, while volatile anesthetics cause a dose-dependent increase in cerebral blood flow, similar circumstances may decrease blood flow to the liver and kidneys (Gelman et al., 1984a, 1984b; Miller et al., 2014). Temporal changes in cardiovascular function have also been reported in a variety of animals during inhalation anesthesia (Vatner and Smith, 1974; Dunlop et al., 1987; Steffey et al., 1987a, 1987b, 1987c, 1987d).

Cardiac Rhythm and Catecholamines

Inhalation anesthetics may increase the automaticity of the myocardium (Price, 1966). This effect is exaggerated by adrenergic agonists (Katz and Epstein, 1968). N-alkane anesthetics such as halothane sensitize the heart to arrhythmogenic effects of catecholamines (Raventos, 1956; Joas and Stevens, 1971; Munson and Tucker, 1975; Johnston et al., 1976; Eger, 1981; Weiskopf et al., 1989; Moore et al., 1993; Navarro et al., 1994; Miller et al., 2014); this side effect is not apparent with conventional ether agents.

Liver

Hepatocellular injury may result as a general consequence of a reduction in hepatic blood flow or by direct volatile anesthetic agent toxicity. The chlorinated methanes, including chloroform, remain a classic research model for induction of hepatic injury (Plaa, 2000). However, of the contemporary volatile agents, liver injury is occasionally reported with halothane (Pohl et al., 1989; Kenna and Jones, 1995). Newer ether-type anesthetics have much less pronounced effects on hepatic blood flow (Merin et al., 1991; Bernard et al., 1992; Crawford et al., 1992; Frink et al., 1992b; Hartman et al., 1992), and isoflurane and desflurane undergo comparatively trivial biotransformation (Holaday et al., 1975; Ghantous et al., 1991; Njoku et al., 1997; Eger et al., 2002).

Gastrointestinal

Although inhaled agents are commonly associated with postanesthetic nausea and vomiting in people, emesis in the recovery period appears uncommon in most domestic animals.

Kidneys

All of the volatile anesthetics reduce renal blood flow and glomerular filtration rate in a dose-related manner (Mazze et al., 1974; Cousins et al., 1976). Decreased urine output during inhalation anesthesia may be a consequence of decreased renal perfusion, but it can also be associated with extracellular fluid accumulation caused by the inhaled anesthetics (Brauer et al., 2002; Connolly et al., 2003; Weiss and Pizov, 2003). A transient increase in serum urea nitrogen, creatinine, and inorganic phosphate also may accompany inhalation anesthesia, especially if prolonged (Steffey et al., 1979, 2005a, 2005b;

Steffey and Howland, 1980; Eger, 1981). In most cases the effects of inhalation anesthesia on renal function are rapidly reversed after anesthesia. Beyond these general responses, methoxyflurane is notable with regard to its potential for causing nephrotoxicity (Mazze et al., 1971, 2006).

Skeletal Muscle

The volatile anesthetics are associated with some small amount of skeletal muscle relaxation, likely a direct result of their CNS depression and depressant calcium channel effects. They also enhance the muscle relaxation induced by the nondepolarizing neuromuscular blocking drugs that are sometimes used as adjuvants in the anesthetic management of patients.

Malignant hyperthermia is life-threatening pharmacogenetic myopathy involving a defect of the ryanodine (or sometimes dihydropyridine) receptor in the sarcoplasmic reticulum (Lanner et al., 2010; Hopkins, 2011; Bannister and Beam, 2013). It is most commonly found in certain pig breeds (Landrace, Pietrain, Poland China, Yorkshire, and Duroc) and in certain human familial lines (Fujii et al., 1991), although genetic mutations associated with the disease have been reported in other domestic species including dogs (Short and Paddleford, 1973; Roberts et al., 2001) and horses (Aleman et al., 2004, 2005). While historically associated with halothane anesthesia (Denborough et al., 1962), all of the modern volatile agents including enflurane (McGrath et al., 1981), isoflurane (McGrath et al., 1981; Boheler et al., 1982), sevoflurane (Shulman et al., 1981), and desflurane (Wedel et al., 1991) can trigger malignant hyperthermia in susceptible individuals. In contrast, nitrous oxide has little or no malignant hyperthermia-triggering effect (Reed and Strobel, 1978; McGrath et al., 1981).

Unless confirmed otherwise, animals related to individuals with known or suspected malignant hyperthermia, including pigs derived from herds reporting the presence of porcine stress syndrome or pale soft exudative pork, are themselves at risk for malignant hyperthermia and should be managed accordingly. The resultant syndrome of malignant hyperthermia is characterized by muscle rigidity, a rapid rise in body temperature, large consumption of oxygen, and consequent production of CO_2. Death rapidly ensues in many cases unless very aggressive therapy is instituted. No volatile anesthetics should be administered in these patients, and anesthetic machines potentially leaching trace vapors should either be avoided or activated carbon filters be used to prevent breathing of triggering agents (Birgenheier et al., 2011). Dantrolene (Harrison, 1975) or azumolene (Dershwitz and Sreter, 1990) are the treatments of choice for a fulminant malignant hyperthermia episode.

Actions by Agent

Additional actions by agent will be highlighted in this section. The volatile agents will be reviewed first, with an emphasis on the most common contemporary ether-type anesthetics: isoflurane, desflurane, and sevoflurane. Because of their limited or declining use in research or clinical practice, methoxyflurane, enflurane, and halothane will be discussed only with respect to major advantages or disadvantages compared to the three haloether anesthetics in greatest contemporary use. Additional information about these older agents and others, such as diethyl ether and chloroform, can be found in previous editions of this textbook. Finally, the gaseous agent, N_2O, will be discussed. Although approaching many of the goals of an “ideal” inhaled anesthetic, xenon remains too scarce and expensive for clinical use in veterinary patients. The interested reader is encouraged to consult other references regarding xenon pharmacology (Lawrence et al., 1946b; Lynch et al., 2000; Sanders et al., 2003; Preckel and Schlack, 2005; Bovill, 2008; Giacalone et al., 2013).

Isoflurane

Isoflurane USP (Forane, Aerrane, IsoFlo, Attane), is a halogenated methyl ethyl ether and a structural isomer of enflurane. It is a racemic mixture of 2-chloro-2-(difluoromethoxy)-1, 1, 1-trifluoro-ethane.

Isoflurane was first synthesized in 1965, and its widespread clinical use for human patients began in 1981. At least in North America, it is now the most widely used volatile anesthetic agent in veterinary patients.

CO_2 Absorbent Stability

Isoflurane is degraded by Baralyme, and to a lesser extent by soda lime containing monovalent bases, to produce carbon monoxide. The magnitude of this breakdown is increased by desiccated absorbent and by high concentrations of the volatile agent (Fang et al., 1995). CO_2 absorbents lacking sodium hydroxide or potassium hydroxide cause negligible breakdown of isoflurane (Murray et al., 1999; Stabernack et al., 2000).

Biotransformation

In humans and animals isoflurane resists biodegradation. In humans less than 0.2% of the isoflurane taken up by the body is metabolized (Holaday et al., 1975); this rate of metabolism is far less than for enflurane or sevoflurane (Table 11.8). Both inorganic fluoride and trifluoroacetic acid have been identified as end products of isoflurane metabolism (Njoku et al., 1997), but serum concentrations are small even after prolonged isoflurane administration due to minimal metabolism (Cousins et al., 1973; Dobkin et al., 1973; Mazze et al., 1974). Isoflurane also

Table 11.8 Biotransformation of inhalation anesthetics in humans

Anesthetic	Metabolized (%)	Reference
Methoxyflurane	50	Holaday et al., 1970
Halothane	20–25	Rehder et al., 1967; Cascorbi et al., 1970
Sevoflurane	3	Eger, 1994
Enflurane	2.4	Chase et al., 1971
Isoflurane	0.17	Holaday et al., 1975
Desflurane	0.02	Eger, 1994
Nitrous oxide	0.004	Hong et al., 1980

does not appear to be a mutagen, teratogen, or carcinogen (Eger et al., 1978; Eger, 1981).

Central Nervous System

Isoflurane, unlike its isomer enflurane, has anticonvulsant effects(Koblin et al., 1981). Electrical silence is seen on the EEG at 2 MAC (Clark and Rosner, 1973). However, cats given isoflurane display “sharp waves” (isolated spiking) on the electroencephalogram (EEG). This activity is also seen in cats with halothane, enflurane, and methoxyflurane and may be a species peculiarity (Julien and Kavan, 1974; Kavan and Julien, 1974).

Cardiovascular System

Isoflurane depresses cardiovascular function in a dose-related fashion (Steffey and Howland, 1977; Steffey et al., 1977, 1987a; Eger, 1981; Ludders et al., 1989) that is modified in magnitude by anesthetic duration (Dunlop et al., 1987; Steffey et al., 1987c). The magnitude of its effect on arterial blood pressure is similar to halothane, although with isoflurane the cause is more related to a decrease in the systemic vascular resistance. Also like halothane, it decreases cardiac contractility and stroke volume, resulting in a decrease in cardiac output. However, results of studies in a number of species indicate that isoflurane, especially at light and moderate levels, affects cardiac output less than halothane does. Isoflurane thus offers a wider margin of safety compared to halothane.

Heart rate tends to be better maintained during isoflurane and remains relatively constant over a range of alveolar isoflurane doses. Heart rhythm is usually little affected by isoflurane, and the incidence of dysrhythmias after injection of vasoactive substances (including catecholamines) is substantially reduced in comparison to halothane (Joas and Stevens, 1971; Tucker et al., 1974; Johnston et al., 1976; Bednarski et al., 1985).

Respiratory System

Isoflurane depresses respiration and increases P_aCO_2 (Steffey and Howland, 1977, 1980; Steffey et al., 1977; Eger, 1981; Miller et al., 2014). The magnitude of depression is dose and time related and is at least equal to or often greater than that caused by halothane under similar conditions (Cromwell et al., 1971; Steffey and Howland, 1977; Eger, 1981; Steffey et al., 1987c; Brosnan et al., 2003b; Miller et al., 2014). In some species, like the horse, during light and moderate levels of anesthesia respiration is characterized by large tidal volume and low breathing rate (Steffey et al., 1977; Brosnan et al., 2003b).

The alveolar concentration that causes apnea is 2.5 MAC for the dog (Steffey and Howland, 1977) and 2.3 MAC for the horse (Steffey et al., 1977).

Desflurane

Desflurane USP (Suprane), formerly known as I-653, is a polyfluorinated methyl-ethyl ether. It is a racemic mixture of 2-(difluoromethoxy)-1,1,1,2-tetrafluoro-ethane.

Although first synthesized in the 1960s along with similar agents such as enflurane and isoflurane, desflurane was not actively investigated for clinical use at that time because it was difficult to produce and had a boiling point only slightly above room temperature (Eger, 1993). Its first reported use in humans was in 1990 (Jones et al., 1990a, 1990b).

Desflurane has a high vapor pressure (Table 11.2) and requires temperature-controlled, pressurized vaporizer for predictable delivery (Andrews et al., 1993). It has a very low solubility in blood (Table 11.3) (Eger, 1987; Soares et al., 2012), and other tissues (Yasuda et al., 1989), contributing to greater precision of control over the maintenance of anesthesia and a very rapid emergence from anesthesia (Eger and Johnson, 1987; Yasuda et al., 1989, 1990, 1991; Jones et al., 1990a; Barter et al., 2004; Steffey et al., 2005b, 2009).

CO_2 Absorbent Stability

Desflurane is degraded by Baralyme or soda lime containing monovalent bases to produce carbon monoxide. The magnitude of this breakdown is increased by desiccated absorbent and by high concentrations of the volatile agent (Fang et al., 1995). CO_2 absorbents lacking sodium hydroxide or potassium hydroxide cause negligible breakdown of desflurane (Murray et al., 1999; Stabernack et al., 2000; Coppens et al., 2006).

Biotransformation

Desflurane resists degradation by the body to a greater degree than any of the other volatile anesthetics (Koblin et al., 1988; Jones et al., 1990c; Koblin, 1992); actual amounts of degradation are too small to measure accurately. Direct renal toxicity has not been reported, direct hepatic toxicity caused by trifluoroacetylation of liver proteins is rare (Njoku et al., 1997; Anderson et al., 2007).

Central Nervous System

Desflurane is less potent than other contemporary volatile agents (Table 11.7). For example, MAC for the dog, horse, and pig is 7.2%, 8.1%, and 10.0%, respectively (Doorley et al., 1988; Eger et al., 1988; Steffey et al., 2005b).

Desflurane causes dose-related depression of EEG activity comparable to effects seen with an equipotent dose of isoflurane (Rampil et al., 1988, 1991). Epileptiform EEG activity is not reported.

Cardiovascular System

The cardiovascular actions of desflurane are similar to those of isoflurane (Weiskopf et al., 1988, 1989, 1991; Pagel et al., 1991b; Warltier and Pagel, 1992; Clarke et al., 1996; McMurphy and Hodgson, 1996; Santos et al., 2005; Steffey et al., 2005b). Like isoflurane and halothane, desflurane decreases mean arterial blood pressure and stroke volume in dose-related fashion. But cardiac output during desflurane, as with isoflurane, is better maintained compared to conditions during halothane anesthesia. Heart rate is usually higher and peripheral vascular resistance less with desflurane compared to the other volatile agents (Pagel et al., 1991a). As with other agents, myocardial contractility is depressed in a dose-dependent manner (Pagel et al., 1991b; Boban et al., 1992). However, initial uptake of desflurane can be associated with increased sympathetic tone, resulting in transient increases in heart rate and blood pressure (Ebert and Muzi, 1993; Weiskopf et al., 1994). Desflurane does not predispose the heart to ventricular arrhythmias, nor does it sensitize it to arrhythmogenic effects of epinephrine (Weiskopf et al., 1989; Moore et al., 1993).

Respiratory System

Desflurane, like other contemporary volatile anesthetics, causes a dose-related respiratory depression (Lockhart et al., 1991a; McMurphy and Hodgson, 1996; Santos et al., 2005; Souza et al., 2005; Steffey et al., 2005b). Its effects in this regard in humans are most comparable to those of enflurane (i.e., more depressing than isoflurane). Apnea occurs in pigs at alveolar desflurane concentrations between 1.2 and 1.6 MAC, while the apneic threshold in dogs is 2.38 MAC (Warltier and Pagel, 1992).

Sevoflurane

Sevoflurane USP (Ultane, Sevoflo), a polyfluorinated methyl-isopropyl ether, was synthesized in the early 1970s, and its characteristics were first described in 1975 (Wallin et al., 1975). It is 1, 1, 1, 3, 3, 3-hexafluoro-2-(fluoromethoxy)-propane which lacks a chiral center. Because sevoflurane was difficult and expensive to synthesize at the time and was known to be degradable, it was not introduced until the late 1980s – first in Japan (Doi and Ikeda, 1987b; Katoh and Ikeda, 1987) and then in the USA in 1995 (Callan, 2012). Sevoflurane formulations containing low concentrations of water may be subject to Lewis-acid degradation in some vaporizers, resulting in formation of hydrofluoric acid (Kharasch et al., 2009).

The blood solubility of sevoflurane is less than isoflurane but, in most species, is greater than desflurane (Wallin et al., 1975; Strum and Eger, 1987; Soares et al., 2012). Compared to other inhaled agents, sevoflurane provides a relatively rapid anesthetic induction and anesthetic recovery (Wallin et al., 1975; Sarner et al., 1995; Hikasa et al., 1996; Johnson et al., 1998).

CO_2 Absorbent Stability

As with other modern ether-type anesthetics, sevoflurane is capable of producing small amounts of carbon monoxide when exposed to Baralyme or older soda lime formulations containing monovalent bases. However, these absorbents also cause sevoflurane breakdown into 1, 1, 3, 3, 3-pentafluoro-2-(fluoromethoxy)-1-propene, also known as compound A (Wallin et al., 1975; Strum et al., 1987; Liu et al., 1991; Frink et al., 1992a), a nephrotoxin that is lethal in 50% of rats (LD_{50}) at a concentration of 400 ppm (Hikasa et al., 1996). In addition, sevoflurane in dry soda lime containing KOH can undergo a Cannizzaro reaction to form methanol and formic acid, and ultimately produce formaldehyde, a respiratory irritant (Funk et al., 1999; Coppens et al., 2006). Fortunately, sevoflurane is stable in newer formulations of CO_2 absorbents that lack monovalent bases (Murray et al., 1999; Stabernack et al., 2000; Coppens et al., 2006).

Biotransformation

Like all fluorinated volatile anesthetics, sevoflurane is biotransformed to organic and inorganic fluoride metabolites (Kharasch, 1995). The in vitro rate of defluorination of sevoflurane is about the same as for methoxyflurane (Cook et al., 1975a, 1975b). In vivo, however, the serum F^- concentration associated with sevoflurane is much less than with methoxyflurane (Cook et al., 1975b; Holaday and Smith, 1981). Likely this difference is related to sevoflurane's reduced tissue solubility. Sevoflurane defluorination is increased by prior induction of microsomal enzymes with drugs such as phenobarbital (Cook et al., 1975a). Serum F^- concentrations from anesthetized horses are similar to those from humans under similar conditions (Aida et al., 1996; Driessen et al., 2002; Steffey et al., 2005a).

Central Nervous System

Like desflurane and other commonly used inhalation anesthetics, sevoflurane decreases cerebral vascular resistance and cerebral metabolic rate, and it increases intracranial pressure in a dose-related manner (Manohar and Parks, 1984b; Scheller et al., 1988, 1990). Sevoflurane's cerebral vasodilating potency is considered a little less than isoflurane and desflurane (Matta et al., 1999; Summors et al., 1999; Kaye et al., 2001; Holmstrom and Akeson, 2003). Compared with isoflurane or desflurane, sevoflurane can be associated with epileptogenic EEG activity in patients (Iijima et al., 2000; Voss et al., 2006), but sevoflurane is not commonly associated with gross motor evidence of seizure activity, at least in dogs (Wallin et al., 1975; Scheller et al., 1990).

Cardiovascular System

Sevoflurane's actions on the circulatory system are qualitatively and quantitatively similar to those of isoflurane (Manohar and Parks, 1984b; Bernard et al., 1990; Eger, 1994; Lerman et al., 1994; Ebert et al., 1995a, 1995b; Pypendop and Ilkiw, 2004). Sevoflurane does not increase the arrhythmogenicity of the heart (Wallin et al., 1975), and the arrhythmogenic dose of epinephrine in dogs anesthetized with sevoflurane is similar to that during isoflurane anesthesia (Hayashi et al., 1988).

Respiratory System

Sevoflurane does not elicit as much airway irritation, coughing, or breath holding as other halogenated ether anesthetics in people, and so is the potent volatile agent most commonly selected for inhaled anesthetic induction (Doi and Ikeda, 1993; TerRiet et al., 2000). However, differences between agents in the quality of anesthetic induction – aside from speed – are less apparent in domestic animals.

In humans, sevoflurane produces less respiratory depression than other modern halogenated ethers at light anesthetic planes, with arterial $P\text{CO}_2$ in spontaneously ventilating patients approximating values observed during equipotent doses of halothane anesthesia. However, respiratory depression at 1.4 times MAC, representing a moderate-to-deep anesthetic plane, is greater with sevoflurane than halothane (Doi and Ikeda, 1987b; Green, 1995).

In horses, sevoflurane tends to depress ventilation more than either isoflurane or halothane, even at light-to-moderate anesthetic planes (Grosenbaugh and Muir, 1998; Brosnan et al., 2012). More potent respiratory depression can delay anesthetic washout (Brosnan et al., 2012), resulting in similar recovery times in horses recovering from isoflurane or sevoflurane, despite the latter's much lower blood–gas partition coefficient (Soares et al., 2012).

Halothane

Halothane USP (Fluothane), is a polyhalogenated alkane first introduced into clinical practice in 1957 (Brennan et al., 1957). It is a racemic mixture of 2-bromo-2-chloro-1, 1, 1-trifluoro-ethane.

Advantages

At most clinically relevant equipotent doses, respiratory rate and ventilation are better maintained with halothane during spontaneous breathing compared with any of the modern halogenated ether-type anesthetics. In otherwise unmedicated healthy dogs and horses, the alveolar halothane concentration that is associated with complete respiratory arrest is 2.9 MAC (Regan and Eger, 1967) and 2.6 MAC (Steffey et al., 1977), respectively. Halothane also does not cause airway irritation; however, sevoflurane shares this property and consequently has replaced halothane use as an inhalation induction agent (Doi and Ikeda, 1993).

Disadvantages

Following are some undesirable features of halothane, many of which have contributed to its declining use and availability:

- Pharmacokinetics: Halothane has a much greater blood : gas partition coefficient than modern haloethers; hence, recovery from anesthesia is prolonged (Whitehair et al., 1993; Polis et al., 2001).
- Stability: Halothane requires a thymol preservative that can accumulate in vaporizers (Rosenberg and Alila, 1984).
- Biotransformation: As much as 40% of halothane undergoes liver metabolism, resulting in production of trifluoroacetic acid (Van Dyke and Wood, 1975). Other metabolites resulting from oxidative metabolism via the cytochrome P450 pathway are inorganic chloride (Cl^-) and to a lesser extent bromine (Br^-). In humans, Br^- from halothane breakdown will induce headache, ataxia, lethargy, and EEG alterations (Tinker et al., 1976).
- Cardiovascular: Halothane decreases myocardial contractility, cardiac output, and blood pressure more than equipotent doses of modern haloethers (Steffey et al., 1974b, 1974c; Merin et al., 1977; Steffey and Howland, 1980; Ingwersen et al., 1988; Grandy et al., 1989; Pagel et al., 1991a). It also predisposes the heart to premature ventricular extrasystoles in the presence of exogenous or endogenous catecholamines (Purchase, 1966; Joas and Stevens, 1971; Munson and Tucker, 1975; Johnston et al., 1976).
- Hepatic: Halothane decreases hepatic blood flow more than ether anesthetics and is commonly associated with an increase associated with an increase in hepatocellular leak enzymes, although patients are not typically symptomatic for hepatic injury (Gopinath et al., 1970; Gopinath and Ford, 1976; Ross and Daggy, 1981; Harper et al., 1982a, 1982b; Shingu et al., 1982a, 1982b; Engelking et al., 1984; Steffey et al., 1993). Infrequently, the metabolite trifluoroacetic acid can

form trifluoroacetylated protein adducts in hepatocytes and stimulate a severe, and often fatal, immune reaction known as “halothane hepatitis” (Satoh et al., 1985; Hubbard et al., 1988; Kharasch et al., 1996; Njoku et al., 1997). Similar hapten immune reactions are possible, but much rarer, with the modern ether agents (Lewis et al., 1983; Carrigan and Straughen, 1987; Martin et al., 1995; Njoku et al., 1997).

Methoxyflurane

Methoxyflurane, USP (Metofane, Penthrane), is a polyhalogenated methyl ethyl ether that was first synthesized in 1958 and introduced clinically a few years later (Artusio et al., 1960; Mazze, 2006). Methoxyflurane is 2, 2-dichloro-1, 1-difluoro-1-methoxy-ethane which lacks a chiral center.

Advantages

Modern haloethers can cause hyperalgesia at low concentrations. In contrast, methoxyflurane is analgesic even at subanesthetic doses (Romagnoli and Korman, 1962; Torda, 1963).

Disadvantages

Following are some of the undesirable effects of methoxyflurane:

- Pharmacokinetics: Methoxyflurane has a very high blood : gas partition coefficient (Soares et al., 2012), resulting in a very slow onset of general anesthesia and very prolonged recoveries (Stoelting and Eger, 1969a).
- Biotransformation: As much as 70–80% of body methoxyflurane is metabolized, a process that can continue for many days after recovery due to the high tissue solubility of the agent (Holaday et al., 1970; Mazze and Cousins, 1974; Yoshimura et al., 1976). Metabolites include fluoride ion, dichloroacetic acid, and methoxyfluoroacetic acid.
- Renal: Methoxyflurane metabolites can cause vasopressin-resistant polyuric renal failure (Crandell et al., 1966; Mazze et al., 1971; Mazze, 2006).

Enflurane

Enflurane USP (Ethrane, Compound 347), was synthesized in 1963, introduced for clinical trial in human patients in 1963, and released for general clinical human use in 1972 (Dobkin et al., 1968). A structural isomer of isoflurane (Figure 11.1), enflurane is a racemic mixture of 2-chloro-1-(difluoromethoxy)-1, 1, 2-trifluoro-ethane.

Disadvantages

Following are some undesirable features of enflurane:

- Pharmacokinetics: The enflurane blood : gas partition coefficient is greater than isoflurane, resulting in less desirable kinetics (Eger and Eger, 1985; Carpenter et al., 1986b).
- Central nervous system: Enflurane causes epileptiform activity on the electroencephalogram at moderate levels of anesthesia and can cause seizures (Joas et al., 1971; Neigh et al., 1971; Julien and Kavan, 1972; Clark and Rosner, 1973; Klide, 1976; Steffey et al., 1977; Steffey and Howland, 1978a; Bassell et al., 1982).

The Gaseous Anesthetic: Nitrous Oxide

Nitrous oxide USP (N_2O), is a colorless, nonirritant, slightly sweet-smelling, nonflammable gas. Nitrous oxide is commercially available as a gas stored in steel cylinders at a pressure of about 750 psi or nearly 50 atmospheres. It has many desirable properties, including low blood solubility (Table 11.3), limited cardiovascular and respiratory system depression, and minimal toxicity (Eger, 1985). A combination of rapid kinetics and high inspired fractions (due to low potency) allow anesthetists to take advantage of a “concentration effect” and “second gas effect,” but also may create tympany of gas filled spaces and “diffusion hypoxia” during recovery. The reader is referred elsewhere for more information on these topics (Eger, 1974, 1985).

Biotransformation

Nitrous oxide is metabolized (reductive pathway) by intestinal anaerobic bacteria to molecular nitrogen (N_2) and free radicals (Hong et al., 1980; Linde and Avram, 1980). Unlike other inhalation anesthetics, N_2O is not directly metabolized by animal tissues.

Central Nervous System

Nitrous oxide is not a potent anesthetic (Table 11.7) and under ambient conditions will not anesthetize a fit, healthy individual. Consequently, to get important benefits of N_2O it is necessary to use it in high inspired concentrations but at the same time remembering that as the concentration of N_2O is increased there is a change in the proportion and partial pressure of the various other constituents of the inspired breath, notably O_2. Consequently, to avoid hypoxemia, 75% of the inspired breath is the highest concentration that can be safely administered (at sea level to healthy individuals, less at altitude or in the face of, especially, cardiopulmonary disease). The potency of N_2O in animals important to clinical veterinary medicine is only about one-half the anesthetic potency of that found for humans. Thus, the value of N_2O in veterinary clinical practice is further compromised. When used, it serves as an anesthetic adjuvant; that is, it is used in conjunction with an injectable and/or another inhalation anesthetic. Since its depression of other vital organs such as the heart, lungs, kidneys, etc., is small in comparison, its purpose in this case is to reduce the amount of the primary, more potent inhaled or injectable anesthetic drug for anesthesia and thereby lessen overall harmful effects on vital organ function.

The effects of N_2O on the EEG are similar to those produced by the volatile anesthetics. At low subanesthetic levels (about 30%, inspired), N_2O increases EEG frequency and lowers voltage, and at higher subanesthetic concentrations (e.g., 60%), N_2O increases voltage. Adding N_2O to a light level of anesthesia produced by other drugs tends to increase voltage and decrease frequency (Eger, 1985).

N_2O causes an increase in cerebral blood flow, cerebral metabolic rate, and intracranial pressure. The magnitude of change seems to depend upon whether it is administered alone or in conjunction with other anesthetics. Dramatic increases in intracranial pressure occur in animals when N_2O is used alone (Theye and Michenfelder, 1968; Sakabe et al., 1978; Pelligrino et al., 1984), but these effects may be masked by coadministration of other potent volatile anesthetics at 0.5–1.0 MAC or greater (Reasoner et al., 1990; Roald et al., 1991; Cho et al., 1996). Nitrous oxide can be neuroprotectant under conditions of glutamatergic excitotoxcity and ischemia, but it might also directly cause neuronal vacuolization and cytotoxicity (Jevtovic-Todorovic et al., 1998, 2003a; David et al., 2003; Jevtovic-Todorovic and Carter, 2005).

Nitrous oxide is an analgesic. It stimulates α-adrenergic receptors in the locus ceruleus and opioidergic receptors in the periaqueductal grey (Zuniga et al., 1987; Fang et al., 1997a; Zhang et al., 1999; Sawamura et al., 2000), resulting in loss of inhibition of descending antinociceptive pathways (Sanders et al., 2008a). As an anesthetic adjuvant, N_2O use is associated with a blunting of autonomic responses that otherwise accompany painful surgical stimuli.

Cardiovascular and Respiratory Systems

Under ambient conditions the effects of N_2O on the cardiovascular and respiratory function (other than reducing the inspired O_2 concentration) are small compared to those of the other inhalation anesthetics. Nitrous oxide is a direct myocardial depressant. However, it also causes sympathetic nervous system stimulation, release of catecholamines, and an increase in systemic vascular resistance. The net result is minimal or no effect on systemic blood pressure in most species, and improved arterial pressures in cats (Eisele and Smith, 1972; Steffey et al., 1974a, 1974c, 1975; Steffey and Howland, 1978b; Eisele, 1985; Pypendop et al., 2003).

In some circumstances N_2O may contribute to an increased incidence of cardiac arrhythmias (Liu et al., 1982; Lampe et al., 1990a). There is some evidence to suggest that its use could contribute to an increased incidence of myocardial ischemia, possibly in circumstances where increased sympathetic tone is contraindicated (Philbin et al., 1985; Leone et al., 1988; Nathan, 1988, 1991; Diedericks et al., 1993).

Liver and Kidney

Nitrous oxide has little or no effect on liver or kidney function in patients exposed under most clinical circumstances (Eger, 1985; Lampe et al., 1990b, 1990c). Nitrous oxide interferes with several vitamin B_{12}-dependent reactions. The result is an irreversible inactivation of the enzyme methionine synthase that in turn results in a reduced amount of thymidine, an essential DNA base. The subsequent interference with DNA synthesis prevents production of both leukocytes and red blood cells by bone marrow. Similarly, exposure to N_2O can produce a polyneuropathy ("nitrous oxide neuropathy") that is indistinguishable from that associated with pernicious anemia, that is, subacute degeneration of the spinal cord (Deacon et al., 1980; Eger, 1985; Koblin et al., 1990). The bone marrow changes would be expected to be seen only in the sickest of patients and after about 10 hours or more of N_2O anesthesia (O'Sullivan et al., 1981). The neurological disease is most commonly associated with rare, long-term exposure in a grossly contaminated work environment or with chronic abuse of N_2O (a potential consideration in the management of the veterinary practice) (Layzer, 1978; Layzer et al., 1978; Eger, 1985; Weir et al., 1988).

Trace Concentrations of Inhalation Anesthetics: Occupational Exposure

In 1968, a published 20-year retrospective study (Bruce et al., 1968) suggested that anesthesiologists exhibited a trend toward higher incidences of death from reticuloendothelial and lymphoid malignancies. The possibility that waste inhalation anesthetics could be mutagenic, carcinogenic, and/or teratogenic raised considerable concern. Although chronic exposure to high concentrations of modern inhalants might be genotoxic or reduce fertility (Rowland et al., 1992; Wiesner et al., 2001), evidence from both animal and human studies strongly suggest that there are no significant health risks posed by chronic exposure to low concentrations of waste gases for most individuals (Burm, 2003). Regardless, it remains prudent to minimize unnecessary workplace exposure to all inhaled anesthetics. Additional information on scavenging and trace gas monitoring is available elsewhere (Dorsch and Dorsch, 2007; Tranquilli et al., 2007; Miller et al., 2014).

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12

Injectable Anesthetic Agents

Lysa P. Posner

Injectable anesthetics provide a rapid means of producing sedation or anesthesia in veterinary patients. The four stages of anesthesia used to define central nervous system (CNS) depression are similar in a patient regardless of whether the anesthetic is injected or administered by inhalation. An advantage of injectable anesthetics, particularly when administered intravenously (IV), is the ability to proceed more rapidly through or bypass stage II anesthesia (the excitement stage). This allows for a rapid and smooth induction of anesthesia, which is aesthetically more pleasant for small animals and safer for personnel in large animals.

There is no anesthetic agent that produces ideal anesthesia under all circumstances. When evaluating an injectable anesthetic agent, it is important to evaluate both the pharmacokinetics (absorption, distribution, metabolism, and excretion) and the pharmacodynamics (the behavioral and physiological effects) of a particular drug in order to select an agent most suited to a particular clinical condition, in a particular species.

Indications for Injectable Anesthesia

Intravenous anesthetics produce rapid unconsciousness which is advantageous for the following reasons. Rapid induction of anesthesia allows for prompt intubation and control of the airway. Intubation can prevent aspiration, facilitates assisted ventilation, and the administration of oxygen, with or without an inhalant anesthetic. Injectable anesthetics allow for unobstructed visualization of the upper airway for examination or surgical procedures and well as endoscopic access to the upper and lower airways. Some IV anesthetics are anticonvulsant and can provide rapid control and reduction in CNS activity in seizing patients. Induction of anesthesia with injectable anesthetics is less stressful for most patients compared with an inhalant induction and attenuates the excitement seen during stage II anesthesia. For large animals (e.g., horses), preventing the excitement stage is paramount for patient and medical personnel safety. Total intravenous anesthesia (TIVA) techniques are increasing in popularity because they can decrease the stress response, are associated with better recoveries, provide cardiovascular stability, and maintain cerebral autoregulation better than inhalants, without the need for expensive anesthetic equipment. This is a particularly attractive method when anesthetizing animals in the field.

Intramuscular (IM) injection of anesthetics can be advantageous for fractious animals or for wildlife or exotic animals, where injectable agents can be administered remotely (e.g., dart or syringe pole).

Finally, injectable anesthesia decreases the exposure of people and the environment to inhalant gases.

Disadvantages of Injectable Anesthesia

In general, injectable anesthetic agents have a large volume of distribution and wake-up is associated with redistribution and then metabolism of the drug. Thus, it can be hard to fine-tune anesthetic depth and wake-up compared with the inhalant drugs that are generally excreted unchanged through the lungs quite rapidly. However, some of the currently available intravenous agents (e.g., propofol) have such short durations of action that depth of anesthesia can be readily adjusted by changing the administration rate. These types of drugs have made it possible for safe and satisfactory anesthesia without the use of inhalation agents.

Another potential disadvantage of injectable anesthetics is the potential for human abuse. Therefore, many injectable anesthetic agents, as well as agents commonly used concurrently with them, are classified as controlled substances under the 1970 US Controlled Substances Act. This places restrictions on purchase, storage, record keeping, and use of these drugs. (Proper handling of Controlled Substances is discussed in Chapter 56 of this book).

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Properties of an Ideal Injectable Anesthetic Drug

There is no ideal injectable (or inhalation) anesthetic drug available at this time. One can, however, hypothesize what properties such a drug would have. These can be divided into physiological and pharmacological properties.

Ideal Physiological Properties

The ideal injectable anesthetic agent should produce unconsciousness and amnesia, as well as provide analgesia and muscle relaxation. The ideal drug would produce those qualities while maintaining physiological homeostasis; that is, there should be no adverse changes in cardiovascular, respiratory, gastrointestinal, CNS, or endocrine functions.

Ideal Pharmacological Properties

An ideal injectable anesthetic should have a wide margin of safety (high therapeutic index) in a variety of species. It should have a short duration of action and be noncumulative. The drug should be readily excreted unchanged, ideally by more than one route. A specific and complete reversal agent should be available. The ideal drug would be chemically stable, have a long shelf life, have a physiological pH, have a nontoxic vehicle, and would be inexpensive.

Barbiturate Anesthetics

History

Barbituric acid was first synthesized in 1864 by Adolph von Baeyer, but the compound was considered clinically useless (Short, 1983). In the early 1900s, the barbiturates or drugs derived from barbituric acid were shown to produce sleep and prevent seizures, and an anesthetic revolution took place. Barbiturates are CNS depressants and can be used at increasing doses to produce sedation, hypnosis, anesthesia, coma, and even death (euthanasia solutions). The major uses for barbiturates in veterinary medicine are as induction agents for anesthesia, anticonvulsants, and euthanasia agents.

Although short-acting barbiturates are still commonly used for anesthesia in both veterinary and human medicine worldwide, they are no longer available in the USA. The European distributors of barbiturate anesthetics will no longer sell them to the USA due to objections regarding their use in human executions (NPR, 2014). Barbiturates for use as anticonvulsants and for veterinary euthanasia solutions are still available in the USA. Euthanasia solutions are discussed in Chapter 16.

Classification

Chemistry: Barbiturates are derivatives of barbituric acid, which is a combination of malonic acid and urea (Figure 12.1, barbituric acid). The addition of an alkyl or aryl group at position 5 of the barbituric acid ring imparts CNS depressant effects. Further modification of the molecule by substituting sulfur (thio) for oxygen (oxy), at position 2 of the barbiturate acid ring, increases the lipid solubility (thiobarbiturates vs. oxybarbiturates) (Figure 12.1, thiopental and methohexital). Lipid solubility can also be enhanced by increasing the number of side chains at position 5a and 5b of the barbituric acid ring (Figure 12.1, thiopental). Increasing lipid solubility results in a decreased duration of action (shorter acting), an increased metabolic degradation, and an increased hypnotic potency (Figure 12.1, methohexital). Longer side chains at position 5 increases anticonvulsant activity (Figure 12.1, phenobarbital, pentobarbital).

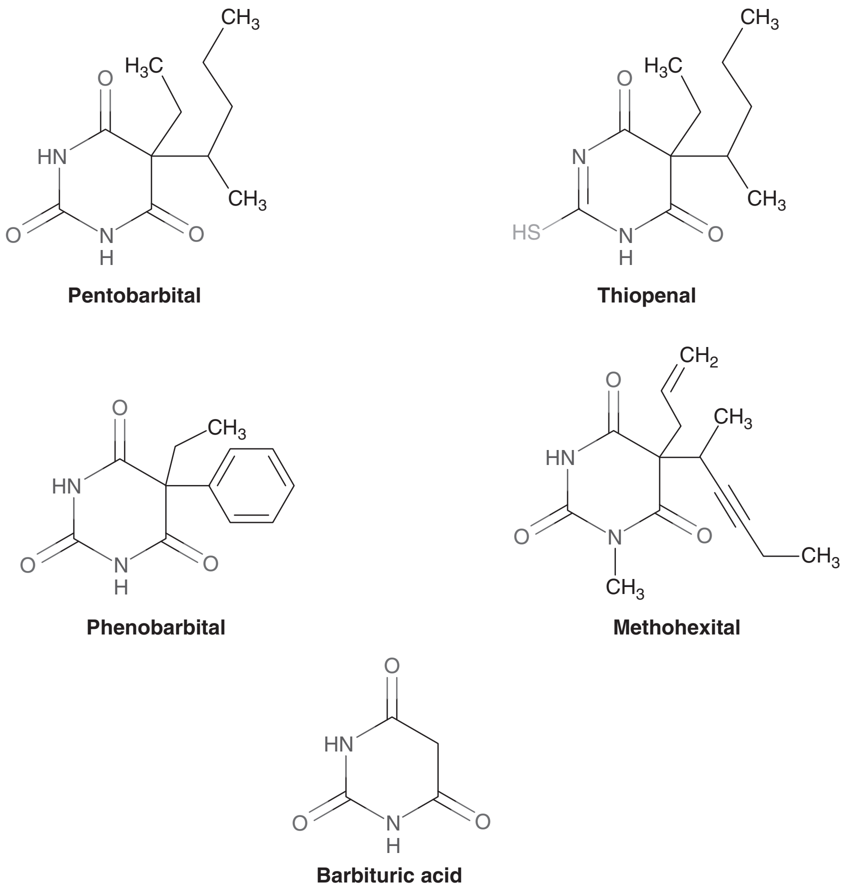
Barbiturates are often classified by either their duration of action (e.g., long, intermediate, short, or ultrashort) or by their chemical structure. The chemical structure (e.g., oxybarbiturates, thiobarbiturates) of barbiturates determine the time of onset and duration of action, which in turn determines their use (Table 12.1).

How supplied: Barbiturates are poorly soluble in water at physiological pH. However, through chemical manipulation, they can be made to form water-soluble salts when added to alkaline aqueous solutions. These salts hydrolyze in water to form highly alkaline solutions with a pH usually between 9 and 10. If the high pH is not maintained (for example if mixed with acidic solutions) precipitation can occur. Thus although barbiturates are chemically acids they are prepared as salts that are bases. The high alkalinity of the solution makes it caustic if given perivascularly (can cause sloughing of tissue) but makes the solution fairly bacteriostatic. Barbiturates are delivered as a racemic mixture with their L-isomers nearly twice as potent as their D-isomers.

Mechanism of Action

Barbiturates are γ-aminobutyric acid ($GABA_A$) receptor agonists which enhance inhibitory neurotransmission. They increase GABA binding (primary inhibitory neurotransmitter) by decreasing the rate of dissociation of GABA from its receptor. Activation of the GABA receptors increases transmembrane chloride conductance, resulting in hyperpolarization of the postsynaptic cell membrane, which causes inhibition of the postsynaptic neuron. Barbiturates also block glutamate

Figure 12.1 Barbiturates and derivatives of barbituric acid.


Pentobarbital

Thiopenal

Phenobarbital

Methohexital

Barbituric acid

binding (main excitatory neurotransmitter) at AMPA receptors.

Barbiturates depress the reticular activating system (RAS), which controls arousal and inhibit the development or spread of epileptiform activity. The RAS system is particularly sensitive to barbiturates (Harvey, 1975) and animals are unable to be aroused or to maintain the wakeful state following administration.

Furthermore, at high doses, barbiturates can decrease transmission of nerve impulses at nicotinic acetylcholine receptors in the neuromuscular junction (Arias et al., 2006). These effects occur because barbiturates decrease the sensitivity of polysynaptic junctions to the depolarizing action of acetylcholine, which results in muscle weakness.

Table 12.1 Classification of barbiturates by type of barbiturate and common use

Drug	Oxygen/ sulfur at position 2	Duration of action	Common use
Phenobarbital	Oxybarbiturate	~12 h	Anticonvulsant
Pentobarbital	Oxybarbiturate	1–2 h	Anesthesia/ euthanasia
Thiopental	Thiobarbiturate	~20 min	Anesthesia
Thiamylal	Thiobarbiturate	~20 min	Anesthesia
Methohexital	Oxybarbiturate	~10–15 min	Anesthesia

Indications

Barbiturates are used in veterinary medicine for rapid induction of general anesthesia, as anticonvulsants, and for euthanasia (for use as anticonvulsants and euthanasia solutions see Chapters 16 and 17).

Specific Barbiturates (Box 12.1)

Thiopental sodium (pentothal sodium, previously used name thiopentone sodium)**:** Thiopental is the most commonly used barbiturate in veterinary medicine worldwide. It is an ultrashort-acting thiobarbiturate and differs from pentobarbital only at the number 2 carbon where a sulfur molecule has replaced the oxygen (Figure 12.1). The drug is prepared as a sterile powder and after reconstitution with an appropriate diluent is administered by the intravenous route. Thiopental sodium is chemically designated sodium 5-ethyl-5-(1-methylbutyl)-2-thiobarbiturate. The drug is a yellowish, hygroscopic powder, stabilized with anhydrous sodium carbonate as a buffer (60 mg/g of thiopental sodium). Thiopental is

Box 12.1 Regulatory information

US Controlled Drug Status
- Thiopental: Schedule III controlled drug
- Phenobarbital: Schedule IV controlled drug
- Pentobarbital: Schedule II controlled drug
- Methohexital: Schedule IV controlled drug

Withdrawal Information for Food Producing Animals
- Thiopental: Extra label use: At least 1 day for meat; 24 hours for milk
- Phenobarbital: no regulatory information available
- Pentobarbital: no regulatory information available

US FDA Veterinary-Approved Products
- Thiopental is approved by the FDA for use in dogs and cats, but no products are currently available in the US.
- Pentobarbital is approved by the FDA for use in dogs and cats
- Pentobarbital: pentobarbital sodium for injection, 64.8 mg/ml, Schering-Plough
- Phenobarbital: none
- Methohexital:none

Human-Approved Products
- Thiopental: Pentothal No longer available in the US
- Pentobarbital: Pentobarbital sodium (Nembutal Sodium), 50 mg/ml, Ovation Pharmacy
- Phenobarbital: Phenobarbital sodium for injection (Generic), 30, 60, and 130 mg/ml
- Methohexital: Methohexital sodium (Brevital), JHP Pharmaceuticals, 500 mg multidose vials.

dispensed as a powder buffered with sodium carbonate which is commonly reconstituted with sterile water or sterile saline. Once reconstituted, thiopental has a pH between 9 and 10, which is caustic if administered perivascularly. The high alkalinity of the solution results in thiopental having a long shelf life, but as the solution ages it becomes less potent (Thurmon et al., 1996). Although supplied in an alkaline pH, thiopental is a weak organic acid with a pK_a value of 7.6, and has a relatively low ionization (39%) at plasma pH (Brandon and Baggot, 1981). The nonionizing moiety is highly lipid soluble, which allows for it to quickly penetrate the blood–brain barrier and thus it is a rapidly acting anesthetic.

In general, the 2.5% solution is used with small animals and the 2.5% or 5% solution is used with larger animals. Thiopental can be mixed with propofol in a 1 : 1 (vol : vol) ratio, which is chemically stable (Paw et al., 1998). The combination of the two GABA agonists produces a synergistic clinical effect and improves the induction and recovery profile of thiopental to more resemble that of propofol (Ko et al., 1999).

Thiopental, like most barbiturates, undergoes extensive liver metabolism.

Thiamylal (Surital)**:** Thiamylal was an ultrashort-acting thiobarbiturate commonly used in veterinary medicine, but is no longer commercially available. Information on this drug can be found in earlier editions of this book.

Pentobarbital sodium (Nembutal sodium)**:** Pentobarbital is a short-acting oxybarbiturate that has been used in veterinary medicine since the early 1930s. Although commonly used in the past for clinical anesthesia, it current use is primarily as a euthanasia solution (see below in this section) and for anesthesia in laboratory rodents. Pentobarbital is effective when given intraperitoneally as well as intravenously and thus can be used successfully in small rodents. It undergoes extensive hepatic metabolism and is totally dependent on the liver for biotransformation and elimination. Animals can show excitement during induction and recovery, which can be prevented by concurrent use of tranquilizers or sedatives.

The duration of action of pentobarbital is much longer (four to eight times) than thiopental in most animals and therefore is therefore less commonly used for anesthesia. However, its long duration of action makes it clinically useful for anticonvulsant treatment.

Euthanasia solutions: Pentobarbital is the primary ingredient in most commercially available euthanasia solutions. See Chapter 16 for more detailed discussion of euthanasia solutions. Lidocaine and phenytoin are often added to increase cardiovascular depression as well as change the solution from a schedule II controlled drug to a schedule III controlled drug. The lethal dose of pentobarbital in the dog is 85 mg/kg orally and 40–60 mg/kg intravenously. Toxicosis, including death, has been reported in dogs fed uncooked meat from a horse euthanatized 8 days previously with pentobarbital (Polley and Weaver, 1977). Cooking does not inactivate pentobarbital in meat, as animals euthanatized with pentobarbital and rendered at a commercial plant showed virtually no degradation of the drug (O'Connor et al., 1985). The recommended dose for euthanasia is 87 mg/kg IV.

Phenobarbital sodium (Luminal, Phenobarbitone)**:** Phenobarbital was first synthesized in Germany in 1912 and was marketed as Luminal. It is a long-acting oxybarbiturate, whose current primary use is as an anticonvulsant or for long-term sedation (e.g., for treatment of animals with strychnine poisoning or tetanus). More information about the anticonvulsant use of phenobarbital is in Chapter 17.

Methohexital sodium (Brevital): Methohexital is a unique oxybarbiturate in that it is ultrashort acting. Methohexital is twice as potent as thiopental (Turner and Ilkiw, 1990) but has a higher incidence of CNS excitatory effects. Methohexital activates abnormal EEG tracings in epilepsy-prone subjects (Musella et al., 1971). Methohexital should not be used in patients with increased CNS excitation (e.g., strychnine poisoning) or with seizure disorders.

Methohexital should be administered intravenously; however, perivascular injection does not produce tissue irritation. Administration of methohexital should be fairly rapid since slow injection rates can be associated with muscular tremors and excitement. Excitement and muscle tremors can be reduced by prior administration of sedatives or tranquilizers.

The duration of action of methohexital is short, with dogs and cats attaining sternal recumbency within 5–10 minutes of administration (Sams et al., 1985). Although methohexital is metabolized four to five times faster than thiopental, awakening is due to redistribution rather than metabolism. In Greyhound dogs, recovery from methohexital anesthesia is significantly faster than from thiopental (Sams et al., 1985) and until the introduction of propofol to veterinary practice, methohexital was considered the induction agent of choice for Greyhound dogs.

Physiological Effects

CNS effects: Barbiturates are administered to produce anesthesia (short-acting barbiturates) or as anticonvulsants (long-acting barbiturates). However, they possess properties that are favorable to cerebral physiology. Barbiturates decrease cerebral blood flow (CBF) (Albrecht et al., 1977) and cause a dose-dependent depression of the electroencephalogram (EEG), eventually resulting in a flat EEG (Kiersey et al., 1951). They decrease cerebral oxygen consumption ($CMRo_2$) particularly in cortical areas by up to 55%, which parallels the depression of the neuronal activity. Metabolic needs of the brain remain constant; only hypothermia will decrease cerebral metabolic needs (Steen et al., 1983). Furthermore, they decrease intracranial pressure (Bedford et al., 1980) and similarly decrease intraocular pressure (Mirakhur and Shepherd, 1985). Since intracranial pressure (ICP) decreases more than mean arterial pressure (MAP), cerebral perfusion pressure is preserved. Thus barbiturates like thiopental are often chosen to anesthetize patients with CNS disease or trauma.

Methohexital, is an ultrashort-acting barbiturate, but is an exception to the cerebro-friendly status of barbiturates. Methohexital has been associated with generalized excitement and activation of epileptic foci (Stoelting, 1999) and is not recommended for patients with a seizure history.

Cardiovascular system: Barbiturates cause changes in the cardiovascular system that is dependent on the dose and the physiological status of the patients. In healthy dogs, there is a decrease in stroke volume and contractility with an increase in heart rate (Turner and Ilkiw, 1990). In the healthy dogs, there is a minimal change to cardiac output as the reflex tachycardia compensates for the decrease in stroke volume, but mild hypotension is often seen.

Although unproved, anecdotal reports suggest that in compromised dogs, barbiturates result in an exaggerated cardiovascular depression. However, healthy dogs that suffered from acute blood loss did not show a depression in cardiovascular parameters (Ilkiw et al., 1991a). Dogs and pigs do, however, need a smaller induction dose following severe hemorrhage (Weiskopf and Bogetz, 1985). Caution should be used when administering barbiturates to patients with dehydration, hypovolemia, anemia, blood loss, or underlying cardiac disease.

Thiopental can precipitate ventricular arrhythmias, particularly bigeminy (Muir, 1977), as well as potentiate epinephrine-induced arrhythmias (Atlee and Malkinson, 1982). Lidocaine can be administered with thiopental. This allows for less thiopental to be administered (reduces cardiovascular depression) and protects against ventricular arrhythmias (Rawlings and Kolata, 1983). Caution should be used when administering thiopental to patients with high sympathetic tone or who are prone to arrhythmias.

There is a dose-dependent effect of the vasomotor center by barbiturates. Rapid IV injection of an induction dose of a thiobarbiturate causes a sharp but transitory fall in arterial pressure because of the high concentration briefly depressing the vasomotor center. Venodilation results in splenic sequestration of red blood cells (RBC) and a concomitant drop in packed cell volume. For this reason barbiturates are often avoided for anesthesia of patients scheduled for a splenectomy (Baldo et al., 2012). Furthermore, the vasodilation contributes to heat loss. With a decline in heat production during anesthesia and an increase in heat loss owing to peripheral vasodilation, anesthetized patients can quickly become hypothermic.

Barbiturates do not block the autonomic responses to pain or intubation and thus should be administered with drugs that will attenuate those responses (e.g., opioids).

Respiratory effects: When used at anesthetic induction doses, barbiturates produce central respiratory depression. There is a decrease in respiratory rate and minute ventilation, with apnea in some patients (Quandt et al., 1998). In addition to causing respiratory depression, the barbiturates decrease the reflex responses to hypercapnia and hypoxemia (Hirshman et al., 1975). Following induction with a barbiturate, patients should be intubated and assisted with ventilation as necessary. The blood concentration inhibiting the respiratory center is considerably less than that arresting the heart, so patients that are apneic may be stable cardiovascularly. The occurrence of bigeminy appears to be decreased in well-oxygenated patients. Oral barbiturates used as anticonvulsants have minimal or no effect on respiratory function, probably due to lower plasma concentrations.

Muscular effects: Barbiturates produce good muscle relaxation and generally produce favorable intubating conditions. Laryngeal reflexes are preserved with barbiturate anesthesia (Jackson et al., 2004) facilitating better evaluation of laryngeal function. The increased sensitivity of laryngeal and bronchial reflexes can lead to laryngospasm (Barker et al., 1992). Although laryngeal reflexes are preserved, all patients induced with a barbiturate should be intubated to protect the airway and support ventilation.

Gastrointestinal (GI) tract: There are no important effects such as diarrhea or intestinal stasis following routine use of thiobarbiturates.

Kidney: The thiobarbiturates have no direct effect upon the kidney, but can decrease urine output because of decreased blood flow and glomerular filtration rate resulting from hypotension and decreased cardiac output. Patients with uremia have decreased plasma binding of barbiturates (more physiologically available drug) and thus need lower induction doses (Ghoneim and Pandya, 1975).

Fetus/neonates: Most barbiturates easily cross the placenta and equilibrium between maternal and fetal circulations is established within a few minutes. While in utero, most fetuses are slightly acidemic when compared to the mother. Since barbiturates are less ionized in an acid environment, it is possible that the neonate will have a greater concentration of active drug. In humans, anesthesia with thiopental for cesarean sections resulted in neonates with depressed reflexes, altered acid–base status, and delayed suckling when compared to epidural anesthesia (Sener et al., 2003). Similarly, neurological function was depressed in puppies following cesarean sections where the dam was administered thiopental (Luna et al., 2004). Pentobarbital has been associated with slow neonatal recovery, delayed nursing, and increased morbidity (Thurmon et al., 1996). Phenobarbital is excreted in the milk, although the amounts are marginal. However, a suckling neonate can ingest the drug passed from the mother (Wong et al., 2008). Because neonates may have impaired clearance compared to an adult, this can potentially lead to adverse effects (sedation and decreased suckling activity).

Miscellaneous: Doses of thiobarbiturates that produce surgical anesthesia depress basal metabolism so that less body heat is produced during anesthesia concurrently with excessive heat loss as a result of vasodilation. It is important that surgical patients anesthetized with barbiturates be kept warm.

The decrease in vasomotion associated with barbiturates also leads to splenic sequestration of RBCs. Since the spleen can become quite large and a significant amount of blood and be stored in the spleen, barbiturates are not recommended for patients having a splenectomy.

The “glucose effect” is a term used to describe a reanesthetizing action seen in animals recovering from barbiturate anesthesia that are administered glucose solutions. Glucose decreases microsomal metabolism and thus can decrease barbiturate metabolism (Peters and Strother, 1972). A similar event can occur with administration of epinephrine; however, little clinical effect is seen at routine barbiturate doses (Hatch, 1966).

Analgesia: Barbiturates do not provide analgesia. While anesthetized, nociceptive input is not perceived by the brain, but the pain pathways are activated. In patients where pain will continue following anesthesia, alternative analgesics must be used. At subanesthetic doses, barbiturates may cause hyperalgesia (Ewen et al., 1995), but this effect is controversial and probably not clinically relevant when barbiturates are used as induction agents.

Pharmacokinetics

Thiopental: Thiopental is highly protein bound (>70%) (Brandon and Baggot, 1981) and has a pKa near 7.6. After IV administration, thiobarbiturates are initially present in high concentrations in highly perfused tissues (e.g., the brain), resulting in rapid induction of general anesthesia. Thiobarbiturates then redistribute to the moderately perfused body tissues (e.g., muscle). This redistribution to moderately perfused tissues decreases the concentration in the plasma in brain tissues allowing the animal to regain consciousness. Further redistribution to adipose tissue from both highly and moderately perfused tissues results in the complete recovery from thiobarbiturate anesthesia. Animals with lower percentages of fat and/ or muscle tissue have a smaller percentage of area for the barbiturates to redistribute to. Thus normally lean patients (e.g., Sighthounds), neonates, or cachetic patients might have prolonged recoveries from thiobarbiturates. Similarly, since awakening depends of redistribution, repeated dosing is not recommended because of cumulative effects and prolongation of recovery.

Pharmacokinetics of thiopental in rabbits, sheep, and dogs showed an central volume of distribution of 38.6 ± 10 ml/kg, 44.5 ± 9.1 ml/kg, and 38.1 ± 18.4 ml/kg, respectively (Ilkiw et al., 1991b). Elimination half-life was longest in the sheep (251.9 ± 107.8 min), shorter in the dog (182.4 ± 57.9 min), and shortest in the rabbit (43.1 ± 3.4 min) (Ilkiw et al., 1991b).

The activity of a drug is dependent on the percentage of the drug that is unbound and unionized. Since thiopental is an acid it is nonionized in an acid environment. It is also highly protein bound. Many ill patients are both acidemic and hypoproteinemic and thus may have greater percentage of active drug available for pharmacological activity. Thus, the therapeutic index may be smaller in ill patients, making them easier to overdose.

Phenobarbital: Phenobarbital is readily absorbed from the GI tract with bioavailability ~90% in dogs (Pedersoli et al., 1987) and ~99% in horses (Ravis et al., 1987). Pharmacokinetics of an IV bolus dose of phenobarbital in horses showed a volume of distribution at steady state of 0.803 ± 0.07 l/kg; terminal phase elimination of 18.3 ± 3.65 hours; and total body clearance 30.8 ± 6.2 ml/kg/h (Duran et al., 1987). In the dog, volume of distribution is ~0.7 L/kg; the mean half-life was 92.6 ± 23.7 hours; mean total clearance 5.6 ± 2.31 ml/h/kg (Pedersoli et al., 1987). In the goat, the volume of distribution for pentobarbital (30 mg/kg) administered intravenously is 0.72 l/kg; the first-order disappearance rate kinetic constant is 0.76 hour and the half-life is 0.91 hour (Boulos et al., 1972).

Phenobarbital: In dogs, the elimination phase half-life of pentobarbital is 8.2 ± 2.2 hours. The steady-state volume of distribution is 1.08 ± 0.21 liters/kg and the elimination clearance is 0.0013 ± 0.0004 liters/min (Frederiksen et al., 1983). It is 35–45% protein bound. Comparatively, ruminants, especially sheep and goats, metabolized pentobarbital much faster; elimination half-life in goats is ~1 hour compared to ~8.0 hours in dogs.

Metabolism

Barbiturates are almost completely biotransformed in the liver and eliminated by the kidney. Most barbiturates are metabolized principally by the hepatic microsomal enzyme system and cause microsomal enzyme induction (CYP450 system). This can result in altered metabolism of other drugs as well of the barbiturate itself if given repeatedly. Neonates, cachetic, or hypothermic animals can have decreased microsomal metabolism and thus these drugs may have a prolonged effect in these animals. Recovery times for patients with hepatic dysfunction can be markedly prolonged, but fairly normal in patients with renal dysfunction.

Although awakening from thiobarbiturates is generally more rapid than from oxybarbiturates (except for methohexital), this is due to rapid redistribution rather than from rapid metabolism.

Greyhounds are deficient in oxidative enzymes needed for metabolism of thiobarbiturates (Robertson et al., 1992). Thus along with having low fat stores, metabolism is extremely prolonged and awakening may be >8 hours. Thiobarbiturates should not be used in Greyhounds.

Tolerance has been reported with multiple or frequent use of barbiturates. It can occur as a sequela to the induction of microsomal enzyme activity (i.e., increased metabolism) or as a general neurological adaptation to chronic barbiturate effects.

Adverse Effects/ Contraindications

Barbiturates can cause sequestration of RBC in the spleen from venodilation. Thus, these drugs are often avoided for a splenectomy. Thiobarbiturates should not be used in Greyhound dogs (see Section Metabolism). Perivascular administration of thiobarbiturates can result in tissue damage and necrosis (sloughing). Infiltration with saline and lidocaine has been recommended for treatment of perivascular injections (Tuohy and MacEvilly, 1982).

Dose

Table 12.2 shows dose ranges to produce anesthesia for common barbiturates in different species. Lower doses are generally used when patients are premedicated with other CNS depressant or have serious concurrent disease and higher doses are generally needed when no other drugs are given. The use of premedicants with barbiturate anesthesia produces a smoother induction and recovery.

The pH of thiopental is ~10 and therefore should only be given IV, as any injection outside of the vein can lead to pain, tissue injury, and skin sloughing. Intraarterially, barbiturates, in particular thiopental, produce spasm of the arterial wall, which can lead to thrombosis and poor perfusion if collateral circulation is not present (e.g., pinnae of ear, digits). Thiopental should not be administered into body cavities.

Thiopental should be administered "to effect." That is, the entire dose is calculated but given slowly until the desired effect is present; usually the end-point is a patient that can be easily intubated. The slower-acting barbiturates need to have a portion of the dose "bolused" to prevent seeing the excitement stage. If thiopental is given after premedication, this quick bolus is unnecessary.

Most barbiturates have a similar but small therapeutic index (lethal dose/ therapeutic dose); however, the shorter-acting barbiturates have fewer postanesthetic complications because of their shorter duration of action.

Increased risk with clinically ill patients: Since World War II, warnings have been issued about the increased mortality with the use of barbiturates in trauma patients

Table 12.2 Doses of common barbiturates in various species

	Thiopental (mg/kg IV)	Pentobarbital (mg/kg IV[a])	Phenobarbital (mg/kg IV)	Methohexital (mg/kg IV)
Dog	8–22	2–30	6.0	3–11
Cat	8–22	2–15	6.0	3–11
Horse	4–15		1.0–10.0	5
Cow	4–22			
Sheep	8–15	20–30		
Goats	8–20	20–30		
Llamas	6–15			
Pigs	5–12	10–30		5–8
Mice		30–90 (IP)		
Rats		20–40 (IP)		
Rabbits	20–50	15–40		10
Chickens	20			
Primates	2–20	37		2

[a] Dose of pentobarbital for euthanasia is 87 mg/kg.
IP, intraperitoneal.

(Halford, 1943). Trauma or critically ill patients are more likely to be acidemic and hypoproteinemic and thus will have an increased fraction of unionized (active) drug available. Uremia can also decrease protein binding and increase the percentage of active drugs. Dogs and pigs with acute blood loss need smaller induction doses (Weiskopf and Bogetz, 1985). Thus these patients are more easily overdosed and care should be used to assure appropriate dosing. However, with proper dose adjustments, the risks for barbiturate use with critically ill patients can be minimized and these drugs can be used safely (Bennetts, 1995).

Duration of action

Duration of action of thiopental in dogs is fairly short with extubation occurring 25.2 ± 15.7 min after an induction dose and ability to walk unaided at 72.8 ± 26.1 minutes (Quandt et al., 1998). Since all of the barbiturates require extensive liver metabolism, it should be expected that neonates and animals with hepatic dysfunction would have a prolonged duration of action. There is also significant individual variation based on sex, weight, nutritional status, body temperature, and breed as well as the type of barbiturate used. For example, thiobarbiturates have a longer duration in Greyhound dogs than in mixed breeds (Robertson et al., 1992). Alternatively, methohexital, an oxybarbiturate, induces a shorter period of anesthesia in the Greyhound dogs than thiopental, a thiobarbiturate (Robertson et al., 1992). Various reasons have been given for the differences in Greyhound dogs as compared to other breeds (Robinson et al., 1986; Court, 1999).

Chloramphenicol can double the duration of action of pentobarbital because it inhibits the microsomal enzymes responsible for metabolism of some barbiturates. Since awakening from thiobarbiturates is primarily due to redistribution rather than metabolism, the concurrent administration of chloramphenicol with thiopental does not prolong awakening when both drugs are used at clinically relevant doses (Reich et al., 2005).

Species Differences

Dogs - sighthounds: Since awakening from anesthesia is due to redistribution of barbiturates to fat and muscle, lean breeds with small fat stores (e.g., Whippet, Irish Wolfhounds) can show a prolonged effect to barbiturates. With little area to redistribute the drug, these breeds also have an increased risk of overdose.

Dogs – Greyhounds: Greyhounds are classified as Sighthounds and are at risks as discussed above. In addition, Greyhounds are deficient in oxidative enzymes needed for metabolism of thiobarbiturates and thus can have extremely prolonged recoveries (Robinson et al., 1986). Although not toxic, the increased risk of overdose coupled with the prolonged recovery makes thiopental not recommended for use Greyhounds.

In Greyhounds, recovery from methohexital anesthesia is significantly faster than from thiopental (Sams et al., 1985). Currently, propofol is more commonly used in Greyhounds for anesthesia than barbiturates.

Ruminants: Barbiturates can be successfully used for anesthesia in ruminants, but because of the propensity for ruminants to regurgitate, the airway needs to be protected by placement of a cuffed orotracheal tube.

In cattle, a congenital condition ("pink tooth") that increases porphyrin concentrations can occur, which can lead to neurological disturbances from demyelination of peripheral and cranial nerves. In the liver, barbiturates stimulate production of an enzyme that increases porphyrin production potentially making the disease worse (Mees and Frederickson, 1975). Animals afflicted with a known or suspected disturbance in porphyrin metabolism should not be administered barbiturates.

Swine: Many pigs may have prolonged recoveries from thiopental administration (~1 hour) versus other animals (~15 minutes). It is possible that redistribution of thiopental to the increased fat stores may contribute to the prolonged effect of the drug. Barbiturates do not trigger malignant hyperthermia.

Horses: The administration of thiopental to horses without prior sedation is not recommended as there is significant excitement and incoordination. Rather, horses should be administered thiopental following sedation with an α_2-agonist (e.g., xylazine) and/or guaifenesin, and/or a benzodiazepine (e.g., diazepam). Induction of anesthesia with thiopental causes horses to become recumbent by the flexion of all four legs, as compared to ketamine where most horses will flex their hind legs first and assume a dog-sitting posture before complete recumbency. Care should be taken to prevent horses from injuring themselves during induction and recovery. Methohexital should not be used alone in horse because excitation during induction and/ or recovery.

Birds: Thiopental has been successfully used in birds interosseously (Valverde et al., 1993), but because of prolonged recoveries and increased mortality it is not recommended.

Reptiles: Barbiturates are metabolized very slowly in reptiles resulting in a prolonged recovery. The ultrashort-acting barbiturate, methohexital, has been used with varying duration of action in snakes (Preston et al., 2010).

Drug Interactions

All CNS depressants (e.g., opioids, α_2-agonists, phenothiazine sedatives) can potentiate the effects of barbiturates, reducing the required induction dose and increasing the risks of physiological side effects. Long-acting barbiturates induce hepatic microsomal enzymes, which increases the metabolism of other drugs metabolized by the same system (e.g., chloramphenicol, beta-blockers, and metronidazole). The induction of microsomal enzymes does not occur after a single dose. Therefore, the likelihood of this occurrence is low with anesthetic uses. However, it is common when repeated oral doses are administered such as when phenobarbital is administered as an anticonvulsant in animals.

The other important interaction that can occur is through microsomal enzyme inhibition. (Anticonvulsant drugs are covered in more detail in Chapter 17.) As discussed in Section Metabolism, drugs known to be CYP450 inhibitors, such as chloramphenicol, can inhibit the metabolism of barbiturates and prolong recovery.

Overdosage/Acute Toxicity

Treatment for an overdose with short-acting barbiturates should center on supportive treatment of respiratory and cardiovascular depression. This may include positive pressure ventilation, fluid therapy, and inotropic support. Clearance of long-acting barbiturates is considerably greater in alkaline than in acid urine. This occurs because alkaline urine produces ionization of the drug and prevents tubular reabsorption. Thus alkalinization of urine with sodium bicarbonate increases the elimination rate and is clinically useful in treatment of intoxication from long-acting barbiturates.

If a known perivascular injection occurs, the area should be infiltrated with saline for dilution and acidification and a local anesthetic to decrease pain.

Propofol

History

Propofol is an intravenous anesthetic used for sedation, induction of anesthesia, or maintenance of anesthesia when administered as a constant rate infusion (CRI). It has been approved for human use since 1989 and is popular in veterinary medicine due to its smooth induction and recovery. It is unusual in that it is supplied in a milky white emulsion that is administered intravenously.

Classification

Chemistry: Propofol (2,6-diisopropylphenol) (Figure 12.2) is an alkyl phenol derivative that is water insoluble but highly lipid soluble. Propofol is chemically described as 2,6-diisopropylphenol and has a molecular weight of 178.27. Propofol is very slightly soluble in water and is therefore formulated as a white, oil-in-water emulsion.

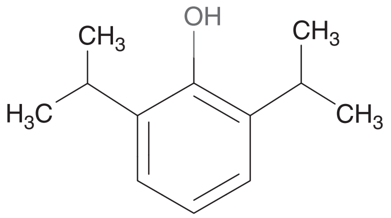


Figure 12.2 Structure of propofol.

How supplied: There are a number different propofol formulations marketed to the human and veterinary markets. Most of the formulations are similar; however, some contain small amounts of EDTA or benzyl alcohol. PropoFlo^{TM} Injection is a sterile, nonpyrogenic emulsion containing 10 mg/ml of propofol suitable for intravenous administration. In addition to the active component, propofol, the formulation also contains soybean oil (100 mg/ml), glycerol (22.5 mg/ml), egg lecithin (12 mg/ml), and oleic acid (0.6 mg/ml) with sodium hydroxide to adjust the pH. The propofol emulsion is isotonic and has a pH of 6.0–9.0. The formulation available contains no preservatives and will support bacterial growth (Wachowski, 1999). Since propofol is an emulsion it should be shaken well before using.

Propofol with extended shelf life, PropoFlo^{TM} 28, is a sterile, nonpyrogenic emulsion containing 10 mg/ml of propofol in a multidose vial for intravenous administration. The name "PropoFlo 28" is used because, compared to previous formulations, it has a 28-day shelf-life once the vial is opened. This is designed to reduce anesthetic waste and no refrigeration is required for the 20-ml and 50-ml vial sizes. Each ml contains 10 mg propofol. The PropoFlo^{TM} 28 emulsion is isotonic and has a pH of 6.0–8.5. PropoFlo 28 is approved for use in dogs, only. The effects of the benzyl alcohol preservative in cats have not been studied and therefore cannot be recommended.

Propoclear® was a propofol formulation that did not use a lipid carrier. The solution was clear and pharmacodynamic effects were similar to standard formulations of propofol. However, the product was associated with increased injection site reactions and was removed from the market (Hill and Williams, 2011).

Mechanism of Action

Propofol produces CNS depression by its effect on the $GABA_A$ receptor (Ying and Goldstein, 2005). Propofol decreases the rate of GABA dissociation from its receptors thus increasing the opening of chloride channels. Increased calcium conductance results in hyperpolarization of postsynaptic cell membranes and inhibition of the postsynaptic neurons resulting in anesthesia and amnesia.

Although shown to be anticonvulsant in veterinary and human patients with variable CNS disease (Brown and Levin, 1998; Steffen and Grasmueck, 2000), the mechanism of action as an anticonvulsant is unknown.

Indications

Clinical uses of propofol include: induction of general anesthesia, short-term sedation, long-term sedation (e.g., ventilator patients), maintenance of anesthesia (via constant rate infusion), and treatment of status epilepticus. Propofol should only be administered intravenously; however, extravasation does not cause skin sloughing as it does with barbiturate anesthetics.

Physiological Effects

CNS effects: Propofol induces CNS depression by enhancing the effects of GABA, an inhibitory neurotransmitter, providing a rapid, smooth induction of general anesthesia.

Propofol produces a favorable neurological status as it decreases ICP, decreases cerebral perfusion pressure, and decreases cerebral metabolic oxygen consumption (Pinaud et al., 1990). Since cerebral metabolic demands are decreased more than blood flow decreases, perfusion is adequate. Intracranial pressure is reduced in patients with both normal and elevated ICP. Propofol has also been shown to maintain cerebral autoregulation in pigs (Lagerkranser et al., 1997) and increase cerebral autoregulation in humans (Harrison et al., 2002).

Similar to its effects on intracranial pressure, propofol causes an acute decrease in intraocular pressure by 30–40% when administered at anesthetic doses (Neel et al., 1995).

Myoclonus has been reported following propofol administration and some have hypothesized that propofol might lower the seizure threshold. However, propofol has been shown to be anticonvulsant and does not produce seizure activity in patients with documented epilepsy (Cheng et al., 1996). Propofol has been used successfully in both dogs and humans for long-term control of status epilepticus (Brown and Levin, 1998).

Following propofol anesthesia, many human patients report a feeling of euphoria as well as having had amorous dreams (Canaday, 1993). Many domestic animals recover from propofol clear headed, coordinated, and seemingly feeling well.

The combination of good CNS perfusion, good quality recovery in patients with CNS disease (Caines et al., 2014), and anticonvulsant properties, makes propofol is a popular anesthetic for patients with CNS disease.

Cardiovascular effects: Induction doses of propofol are associated with systemic hypotension (Reich et al., 2005). Although propofol causes negative inotropy, the decrease in blood pressure is primarily via a decrease in systemic vascular resistance (vasodilation); systemic vascular resistance decreased by 21% in dogs following an induction dose of propofol (5 mg/ml) (Wouters et al., 1995). The decrease in system vascular resistance likely contributes to splenic engorgement and this should be considered in patients anesthetized for splenectomy (Baldo et al., 2012). Following an induction dose of propofol, the heart rate is often decreased or unchanged, even with systemic hypotension (Whitwam et al., 2000). This is contrast to the barbiturates where there is often an increased heart rate in response to the decrease in blood pressure. Therefore a decrease in cardiac output should be expected.

Propofol can enhance the ability of epinephrine to induce cardiac arrhythmias but does not appear to be inherently arrhythmogenic (Kamibayashi et al., 1991).

Respiratory: Apnea is a commonly seen following bolus administration of propofol. The apnea associated with propofol is increased as the rate of infusion is increased. That is, the faster the administration the more likely the incidence of apnea (Musk et al., 2005). Respiratory depression resulting in mild hypercapnia and acidosis are also seen in spontaneously breathing dogs following induction doses (Robertson et al., 1992) and in dogs administered propofol as a constant rate infusion. Patients given propofol should be monitored for hypoxemia and hypercapnia and if necessary the patient should be intubated and provided supplemental oxygen. Propofol should be cautiously in patients where securing an airway may be difficult (e.g., laryngeal masses, guinea pigs, etc.).

Muscular effects: Propofol provides excellent muscle relaxation, but occasionally results in short and long-term myoclonic movements in humans and dogs (Nimmaanrat, 2005). Signs resolve spontaneously, but severity of movement can interfere with surgical procedures. Anecdotal treatment with ketamine at 1 mg/kg IV has resulted in the resolution of signs, whereas treating with benzodiazepines does not appear to affect the myoclonus (author's personal experience). Laryngeal reflexes are decreased and result in a favorable intubating environment, but might increase the risk of aspiration in patients without a protected airway.

Miscellaneous: Propofol crosses the placenta and enter the fetal circulation, but it appears to be readily removed from the fetal circulation after birth and produces minimal effects on healthy, newborn, human infants (Dailland et al., 1989) or puppies (Luna et al., 2004).

Propofol does not cause histamine release (Mitsuhata and Shimizu, 1993) and results in a diminished stress response compared with inhalant anesthetics (Ledowski et al., 2005).

Propofol has antioxidant effects in humans, dogs, and pigs (Aldemir et al., 2001; Allaouchiche et al., 2001; Lee, 2012). The clinical relevance of this activity is unclear.

Propofol has been shown to decrease postanesthetic nausea and vomiting in people at both anesthetic and subanesthetic doses (Apfel et al., 2004); however, this effect has not been documented in domestic animals.

Subanesthetic IV doses of propofol have been shown to decrease the pruritus seen following spinally administered opioids in humans (Horta et al., 2006).

Propofol does not provide any analgesia or produce hyperalgesia as has been reported with barbiturate anesthesia (Wilder-Smith et al., 1995).

Pharmacokinetics

The pharmacokinetics of propofol in dogs was described using a two-compartment open model (Zoran et al., 1993). Initially, the drug is extensively taken up by the CNS, resulting in rapid CNS depression and induction of anesthesia. It is then rapidly redistributed from the brain to other tissues and removed from the plasma by metabolism. Propofol's lipophilic nature results in a large apparent volume of distribution (Vd; 17.9 l/kg in mixed-breed dogs) and steady-state volume of distribution ($\mathrm{Vd}_{\mathrm{ss}}$ 9.7 ml/kg). The initial distribution half-life ($t_{1/2\alpha}$) is short, as is the plasma disappearance ($t_{1/2\beta}$), due to rapid redistribution of the drug from the brain to other tissues and extensive metabolism. The Vd and $\mathrm{Vd}_{\mathrm{ss}}$ are smaller in Greyhounds, 11.2 l/kg and 6.3 ml/kg respectively, which will produce higher concentrations at similar doses compared to other breeds, and there may be slower recoveries compared with other dogs (Zoran et al., 1993). Geriatric dogs (>8.5 years) have also been shown to have a slower clearance than that reported for younger dogs (Reid and Nolan, 1996). Slower systemic clearance may also account for the prolonged effects in Greyhounds and geriatric dogs (see Section Metabolism).

The pharmacokinetics of propofol in cats has not been fully determined; however, cats appear to have a similar response to and dose requirement as dogs for propofol. Interestingly, it has been shown that in cats there is a significant pulmonary uptake of propofol (Matot et al., 1993).

Propofol does not accumulate (noncumulative) after repeated doses and/or prolonged infusions in dogs or laboratory animals (Adam et al., 1980; Mandsager et al., 1995); however, in cats longer constant rate infusions resulted in a longer recovery time (Pascoe et al., 2006).

Metabolism

The total body clearance of propofol is rapid and exceeds hepatic blood flow, suggesting extrahepatic metabolism (Veroli et al., 1992). Indeed, in one study performed in human patients undergoing liver transplantation, the amount of propofol metabolite excreted did not decrease when the liver was excluded from the circulation (Veroli et al., 1992) and the pharmacological effects are not different in patients with cirrhosis (Servin et al., 1990). The site of extrahepatic metabolism is unclear, but pulmonary tissue has been shown to contribute to propofol metabolism in cats (Matot et al., 1993). There may be significant species differences in the site and amount of extrahepatic metabolism.

Although awakening is generally attributed to redistribution, the termination of the effect of propofol is largely attributed to rapid and extensive biotransformation by the liver to inactive metabolites, which are excreted in the urine. In the dog the two major urinary metabolites of propofol are the glucuronide and sulfate conjugates of 4-hydroxypropofol (2,6-diisopropyl-1,4-quinol) (Hay Kraus et al., 2000). The formation of this intermediate derivative appears to be a critical initial step in the biotransformation and elimination of propofol in dog (Hay Kraus et al., 2000). It has also been shown that there are breed differences in metabolic enzyme pathways, which would account for the slower drug clearance seen in Greyhound and geriatric dogs (Reid and Nolan, 1996; Court et al., 1999; Hay Kraus et al., 2000). Although the amount of hepatic glucuronidation has not been studied in cats, there are reports of delayed recovery in cats after prolonged constant rate infusions of propofol, possibly due to the deficiency in cats of enzymes needed for glucuronidation (Pascoe et al., 2006).

Adverse Effects/ Contraindications

Propofol can cause local pain on injection, which is often worse in small peripheral veins. Some anesthetists will preferentially choose larger veins, administer through a running IV line, or administer a small volume of lidocaine IV immediately before administering propofol to prevent the pain on injection.

Propofol without preservative has been associated with increased wound infection. Retrospective analysis of dogs and cats with clean wounds showed an almost four times greater risk of postoperative infection in animals administered propofol (Heldmann et al., 1999). Strict aseptic technique should be emphasized when preparing and administering propofol and unused drug should be discarded within 24 hours (of the nonpreservative formulation). Surgeons performing surgery on patients anesthetized with propofol should be cognizant of this increased risk.

Because of the lipid emulsion, propofol has been suggested as a cause of postanesthetic pancreatitis. Pancreatitis associated with propofol has been reported, albeit rarely, in humans; it has not been reported in veterinary species (Gottschling et al., 2005). Furthermore, it is commonly used in patients with active pancreatitis (Herman et al., 2005).

Propofol syndrome is a rare but often lethal complication following a prolonged continuous administration of propofol. Patients can develop a metabolic acidosis, rhabdomyolysis, kidney injury, cardiac arrhythmias, and cardiac failure. The pathophysiology of this syndrome appears to involve a disturbance of mitochondrial metabolism induced by propofol (Trampitsch et al., 2006). Neonates and septic patients appear to have a greater risk of developing the syndrome. Any patient on a prolonged infusion of propofol should be monitored for these potential adverse signs.

Species Differences

Dogs: Propofol can be used for short or long-term sedation, induction of general anesthesia, and continuous rate infusions. The induction dose required for Greyhounds is not different from that required for mixed-breed dogs; however, the recovery time was longer for Greyhounds (see Section Metabolism).

Cats: Propofol can induce oxidative injuries to feline red blood cells when administered as an induction dose followed by a 30 minutes constant rate infusion. This can result in Heinz body formation, anorexia, diarrhea, and malaise (Andress et al., 1995). However, cats administered induction doses of propofol twice daily for 5 days did not show adverse hematological changes (Bley et al., 2007).

Equine: Propofol should not be used as a sole induction agent in horses as it is associated with excitement. The excitement is likely caused by the long time required to administer the large volume of drug, resulting in the display of stage II anesthesia (excitement stage). Following sedation with an α_{2}-agonist many horses still showed excitement at induction with propofol (Mama et al., 1996). Heavy sedation with guaifenesin has been shown to mitigate the excitement at induction (Brosnan et al., 2011).

The quality of recovery from propofol anesthesia horses is good (Mama et al., 1996; Posner et al., 2013). Although good recovery characteristics are important for horses, the presence of excitement during induction and the prohibitive cost for large patients make it unlikely that propofol will be routinely used in equine patients.

Pigs: Propofol does not trigger malignant hyperthermia in susceptible animals (Fruen et al., 1995). However, good sedation is necessary to provide reasonable venous access. Respiratory depression and apnea have been reported with propofol administration in pigs (Tendillo et al., 1996); thus, propofol should be used with caution unless intubation can be readily accomplished.

Bird: Propofol produces significant respiratory depression in red tailed hawks and great horned owls (Hawkins

Table 12.3 Propofol induction dose and constant rate infusion (CRI) dose for animals

Species	Induction dose (mg/kg IV)	CRI dosage (mg/kg/min IV)	Reference
Dog	3–7	0.2–0.6	
Cat	5–8	0.5–1.0	
Horse – adult	4–8		Mama et al., 1996
Horse – foal	2.0	0.33	
Donkey	2	0.21	
Pigs	2–3	0.1–0.2	Tendillo et al., 1996
Llama	3.3	0.4	del Alamo, 2014
Ferret	2–4		
Rabbit	2–10		

et al., 2003). It is reasonable to presume that all birds may need ventilatory assistance when anesthesia is maintained with propofol. CNS excitement has been noted at recovery (Hawkins et al., 2003).

Fish: Propofol has been used to produce anesthesia in fish both intravenously and via immersion (Fleming et al., 2003; Oda et al., 2014).

Dose

Propofol should be administered slowly to effect. The dose needed will depend on the type and dose of premedication, the health of the patient, and how painful the procedure will be. Premedicants that add CNS depression can decrease induction doses by more than 50%. Time of unconsciousness depends on premedicants and dose of propofol administered. Generally, unconsciousness last from 2–8 minutes. The doses listed in Table 12.3 are provided for the indicated species; however, each patient is different and the total dose should be titrated for a particular patient.

Drug Interactions, Compatibilities, and Incompatibilities

Propofol is compatible with 5% dextrose which can be used to dilute propofol for constant rate infusion. Propofol has been mixed with thiopental in a 1 : 1 solution. The mixture has been shown to be stable and provided smooth inductions that were similar to thiopental but better recoveries (more like propofol) (Ko et al., 1999).

Overdosage/acute toxicity: Accidental overdose of propofol can result in severe cardiovascular and respiratory depression. Treatment is supportive: intubation and ventilation, fluid therapy, positive inotrope, and/or vasoactive drugs. Fortunately, because of rapid recovery, the duration of effect is short.

Regulatory Information

Controlled drug status: Propofol is a nonscheduled (controlled) drug, but has been proposed to be placed in Schedule IV.

Withdrawal times: Withdrawal times have not been established for animals that produce food. However, FARAD recommends at least 72 hours for meat (Lin and Walz, 2014), but could not offer recommendations for milk due to lack of data.

Drug Availability

FDA Veterinary Approved Products

Rapinovet (Schering-Plough Animal Health Corp) 10 mg/ml in 20-ml vials
PropoFlo (Abbot Laboratories) 10 mg/ml in 20-ml vials
PropoFlo 28 (Abbot Laboratories) 10 mg/ml in 20 and 50-ml vials (for dogs only).

Human Products

Diprivan (Zeneca) 10 mg/ml in 20, 50, and 100-ml vials
Propofol (Hospira) 10 mg/ml.

Dissociative Anesthetics

History

Dissociative anesthetics (e.g., ketamine) have been used extensively in veterinary medicine and may still be the most common class of anesthetics in use. The first dissociative anesthetic used was phencyclidine hydrochloride (PCP, “angel dust”), which is no longer commercially available because of the abuse of this drug among humans. Newer phencyclidine derivatives include ketamine hydrochloride and tiletamine hydrochloride. Ketamine is also a drug of abuse in humans (“special-K”) and tiletamine is only marketed in combination with zolazepam under the trade name Telazol® or Zolatel®.

The term dissociative anesthetic originated from the use of ketamine in human patients. When anesthetized with ketamine, patients appeared to feel “dissociated” from or unaware of their environment, but did not always appear asleep (cataleptic). Later it was determined that these drugs did indeed dissociate the thalamocortical and limbic systems causing the change in awareness.

Aside from their use as general anesthetics, dissociative anesthetics are used as analgesics for acute and

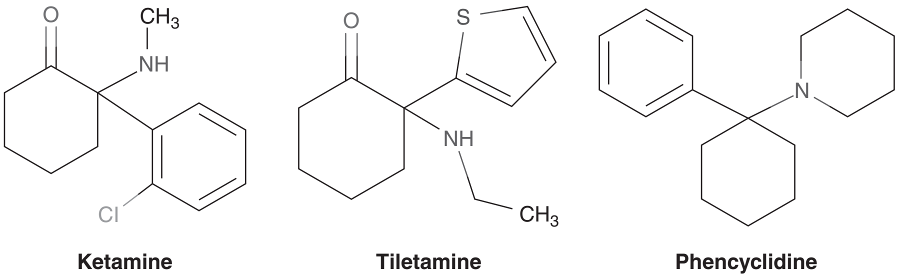


Figure 12.3 Structures of ketamine, tiletamine, and phencyclidine.

chronic pain states and as well as antidepressants (Stahl, 2013).

Classification

Chemistry: (Figure 12.3) Ketamine, 2-(o-chlorophenol)-2-(methylamino)-cyclohexanone hydrochloride, is a phencyclidine derivative that exists as a racemic mixture with the S (+) isomer having greater potency than the R (−) isomer (Casoni et al., 2014). The S (+) isomer produces more analgesia and less emergence delirium than the R (−) isomer.

Tiletamine, 2-[ethylamino]-2-[2-thienyl]-cyclohexanone hydrochloride, is a phencyclidine derivative.

How supplied: Ketamine is supplied as a 10%, aqueous solution (pH 3.5–5.5). It is preserved with benzethonium chloride.

Tiletamine is supplied only in combination with zolazepam as a white powder. The addition of 5 ml diluent produces a solution containing the equivalent of 50 mg tiletamine base, 50 mg zolazepam base, and 57.7 mg mannitol per milliliter. This solution has a pH of 2 to 3.5.

Mechanism of Action

Dissociative anesthetics are noncompetitive antagonists at *N*-methyl-D-aspartate (NMDA) receptors. These drugs prevent the binding of excitatory neurotransmitters, glutamate, and glycine at the NMDA receptor, preventing conduction of ions (Na^+, K^+, and Ca^{2+}). This prevents firing of the second-order neurons and results in depressed activity at the thalamocortical and limbic systems and depression of nuclei in the reticular activating system. Dissociatives also have weak effects at opioid receptors (mu > kappa), monoaminergic receptors, muscarine receptors, and sigma-1 receptors (endoplasmic reticulum calcium channels), but unlike many other induction agents do not interact with GABA receptors (Annetta et al., 2005; Stahl, 2013). Anesthesia with dissociatives produces an altered consciousness or catalepsy.

Unlike other induction agents, dissociatives anesthetics produce analgesia. Analgesia for acute pain is likely at least partially mediated through activation of mu opioids receptors (Sarton et al., 2001). Additionally, the NMDA receptor is also involved in the induction and maintenance of central sensitization in the dorsal horn of the spinal cord (Woolf and Thompson, 1991). Blocking the NMDA receptor with subanesthetic doses of ketamine can interrupt the central sensitization (Correll et al., 2004). Antagonism of the NMDA receptor has also shown great promise in the treatment of refractory depression; however, the mechanism of action as an antidepressant is unknown (Stahl, 2013).

Indications

Dissociative anesthetics can be used for chemical restraint, rapid induction of general anesthesia, and to provide analgesia (for both acute pain as well as interruption of central sensitization). They can be used in most species and can be administered intramuscularly and intravenously.

Physiological/Pharmacodynamic Effects

CNS effects: Based on EEG findings, the antagonism of NMDA receptors produces a dissociation of the thalamocortical and limbic systems (Reich et al., 2005). This produces an altered consciousness or catalepsy, where the patient does not appear asleep, but does not react to noxious or nonnoxious stimuli. Although patient may not look asleep, NMDA antagonists are effective at producing amnesia (Haas and Harper, 1992). When used alone, the dissociative anesthetics rarely produce a surgical depth of anesthesia, but when combined with other CNS depressants produce adequate relaxation and immobility.

The CNS effects of dissociatives are different than most other anesthetics. They increase CBF which is coupled with an increase in cerebral glucose metabolism and oxygen demand ($CMRo_2$) (Dawson et al., 1970). The increased CBF is caused by vasodilation of cerebral vessels and an increase in blood pressure, which results in an increase in ICP. Hypercapnia contributes to the rise in ICP and controlling ventilation can attenuate the rise in ICP. Thus, these drugs should be used with caution in any patient having or suspected of having elevated

ICP, or where cerebral metabolic demands may not be being met. The effects on ICP are also associated with increased intraocular pressure. Ordinarily, this is not a problem, but these agents should not be used in patients with glaucoma.

Ketamine causes the development of EEG patterns that are epileptiform (Kayama, 1982) and is used to elicit more robust seizures during electroconvulsive therapy (Krystal et al., 2003). Interestingly, there is also evidence that ketamine is anticonvulsive (Reder et al., 1980) as well as neuroprotective (Himmelseher and Durieux, 2005). However, most anesthesiologists recommend avoiding ketamine in patients with a seizure history and it should not be used in patients with elevated intracranial pressure.

Dissociative anesthetics cause an increase in norepinephrine via central adrenergic stimulation and a decreased reuptake of norepinephrine, resulting in an increase in sympathetic tone (Stoelting, 1999). The increase in norepinephrine affects all systems under adrenergic control (e.g., cardiovascular, respiratory, endocrine, etc.).

Emergence delirium (e.g., anxiousness, vocalization, thrashing) is a concern whenever dissociative anesthetics are used. Delirium may be merely unpleasant in smaller animals, but can be dangerous when used in larger animals (e.g., horses). Emergence delirium can be attenuated or prevented by administering dissociative anesthetics with sedatives or tranquilizers (e.g., xylazine, diazepam, acepromazine). The R (+) isomer of ketamine is associated with more delirious effects (Stoelting and Hillier, 2012).

Awakening from ketamine is primarily due to redistribution of drug, although some effects are from metabolism.

Cardiovascular system: Ketamine has direct myocardial depressant effects (Diaz et al., 1976) but its administration is generally associated with increase in cardiac output, mean aortic pressure, pulmonary arterial pressure, central venous pressure, and heart rate (Haskins et al., 1985). The stimulatory effects are due to directly stimulating the central adrenergic centers (i.e., increased sympathetic tone) and by inhibiting the neuronal uptake of catecholamines, especially norepinephrine (Annetta et al., 2005). The cardiovascular stimulation is associated with an increase in myocardial work and myocardial oxygen demands (Haskins et al., 1985), thus ketamine should be used with caution in patients with cardiovascular disease that cannot tolerate increased metabolic needs. Although the sympathetic stimulation overrides the direct cardiovascular depressant effects of ketamine (Adams, 1997), patients that are catecholamine depleted (i.e., critically ill) may show more of the cardiodepressive effects (Waxman et al., 1980).

The increased sympathetic tone also may cause tachycardia. Caution should be used with patients that are already tachycardic or dysrhythmic.

Ketamine preserves cardiovascular function and oxygen balance during experimentally produced septic shock (Van der Linden et al., 1990). This cardiovascular sparing effect may be due to the ability of ketamine to block the inflammatory cascade caused by endotoxins that depress cardiovascular function (Taniguchi and Yamamoto, 2005) as well as by increasing levels of norepinephrine that may attenuate the inappropriate peripheral vasodilation seen in septic patients.

Respiratory: Dissociative anesthetics generally have a mild effect on minute ventilation and therefore usually cause a moderate increase in carbon dioxide concentrations. This is in contrast to many other anesthetics that are potent respiratory depressants. However, when other CNS depressants are administered with ketamine, significant respiratory depression can be observed. Ketamine also can cause an *apneustic* pattern of breathing, which is characterized by a rapid sequence of breaths followed by breath holding on inspiration. This occurs more commonly with rapid IV administration of ketamine. Although apneustic breathing is unusual to watch, minute ventilation and carbon dioxide levels are generally within normal limits.

Ketamine has bronchodilating properties and decreases airway resistance (Durieux, 1995). This makes it useful as an induction drug for patients with clinical asthma as well as patients with obstructive airway disease (e.g., chronic obstructive pulmonary disease).

When ketamine is used as a sole anesthetic, pharyngeal and laryngeal reflexes remain active (Robinson et al., 1986). Preservation of these reflexes, however, leads to an increase risk of laryngospasm and coughing secondary to secretions or manipulation in the oropharynx. These complications make ketamine alone a poor drug for use in endoscopy or oropharyngeal surgery. Although laryngeal reflexes are preserved they can be uncoordinated, and thus aspiration is possible. Endotracheal intubation should be used to maintain and protect the airway.

Additionally, ketamine stimulates salivation, which can be copious at times. The administration of an anticholinergic agent (e.g., glycopyrrolate) for its antisialagogue properties can attenuate the amount of salivation. Caution should be exercised when using anticholinergics in patients with small airways as the antisialagogue properties can make for viscous secretions that can occlude the airways or endotracheal tube.

Upper airway muscle tone and reflexes are preserved when dissociatives are used alone. However, since these drugs are generally administered with muscle relaxants, laryngeal control is then diminished or absent.

Endotracheal intubation is recommended for all anesthetized patients to maintain and protect the airway.

Muscular effects: Dissociative anesthetics provide little muscle relaxation and may cause muscle rigidity, myoclonus, and/ or uncoordinated muscle movements. Muscle relaxation is generally provided by the coadministration of sedatives or tranquilizers (e.g., benzodiazepine, α_2-agonists).

Miscellaneous: The appearance of patients anesthetized with dissociative anesthetics is different than with most other anesthetics. Many patients do not close their eyes, can have muscle tone and muscle movement, and often do not look "asleep". It is important to protect the corneas of patients with adequate lubrication and prevent objects from rubbing on the cornea. Coadministration of sedatives or tranquilizers can attenuate these signs.

Analgesia: As described in the Mechanism of Action section, NMDA antagonists such as ketamine produce analgesia. For many years the dogma has been that ketamine provided greater somatic compared with visceral analgesia. However, this view is too simplistic to explain the varied analgesic effects and should be discarded. The mechanism for antinociception is complex and likely involves interactions at more than one type of receptor. Ketamine provides a use-dependent (i.e., works better when the nerves are stimulated) blockade of the NMDA receptor as well as inhibiting neurotransmitter release, both of which has been shown to mediate nociception (Annetta et al., 2005). Analgesia is also likely mediated by the action of ketamine on mu and kappa opioids receptors (Annetta et al., 2005). Opioid receptors extend beyond the CNS and spinal cord and occur in many peripheral tissues. Ketamine produces analgesia at subanesthetic doses for acute surgical pain and have opioid-sparing effects (Annetta et al., 2005).

Additionally, NMDA antagonists at subanesthetic doses are effective at treating chronic pain associated with central sensitization (wind-up pain), neuropathic pain (Guirimand et al., 2000), and likely other types of inflammatory pain. Ketamine by constant rate infusion has been successful in interrupting central sensitization. The duration of the infusion needs to be at least 24 hours, but at times, administration was continued for days to weeks before signs abated (Correll et al., 2004; Sigtermans et al., 2009). When ketamine is used at subanesthetic doses, behavioral changes are rarely seen.

Pharmacokinetics

Pharmacokinetic values for ketamine in various species are summarized in Table 12.4. Both ketamine and tiletamine are highly lipid soluble, so absorption is rapid from the intramuscular route with peak plasma concentration is reached in ~10 minutes (Baggot and Blake, 1976). Ketamine can also be absorbed through oral or rectal mucous membranes. This makes it possible to administer by spraying the drug in the mouth of fractious/feral animals or to administer rectally (Hanna et al., 1988). Cats administered ketamine orally often salivate profusely, presumably from the bitter taste or low pH or both. Cats administered ketamine rectally showed a slow smooth induction, no discomfort from this route of administration and a ~43% availability of drug (Hanna et al., 1988).

Metabolism

Ketamine: In most species, ketamine is biotransformed by the liver by *N*-demethylation to norketamine which then undergoes hydroxylation to form a water-soluble glucuronide derivative that is eliminated in urine (White et al., 1982; Stoelting and Hillier, 2012). Norketamine is an active metabolite of ketamine and has 10–30% activity of the parent drug (Leung and Baillie, 1986; Hanna et al., 1988). In the cat, because of deficiencies in some glucuronidation pathways, ketamine only undergoes transformation to norketamine which is excreted largely unchanged in the urine (Hanna et al., 1988). Since the active metabolites are excreted by the kidneys, decreased renal excretion may prolong the effects of the drug.

Telazol: Telazol is an equal mixture of tiletamine and zolazepam (a benzodiazepine), so the metabolism of this drug needs to be discussed in terms of the two drugs.

Table 12.4 Pharmacokinetics of ketamine in various species

Species	Dose (mg/kg IV)	Distribution phase (min)	Elimination half life (min)	Protein binding %	Clearance (ml/min/kg)	Reference
Dog	15	1.95	61	53.5	32.2	(Kaka et al., 1979)
Cat	25	2.7	78.7	53	21.3	(Hanna et al., 1988)
Horse	2.2	2.9	42	50	26.6	(Kaka et al., 1979)
Calf	5.0	6.9	60.5		40.4	(Waterman, 1984)
Humans	2.2	4.68	130	60		(Domino et al., 1984)

In cats, the duration of effect of zolazepam exceeds that of tiletamine so that, with time, the pharmacodynamic effect is that of the benzodiazepine tranquilization (Telazol Product-Literature, 2017). In dogs, the duration of effect of tiletamine exceeds that of zolazepam so that, with time, the pharmacodynamic effects reflect the dissociative anesthetic, including muscle rigidity, sympathetic stimulation, and emergence delirium (Telazol Product-Literature, 2017). Other species have similar disparities in metabolism and recoveries from Telazol. Pigs awaken slowly and calmly, whereas horses can have an agitated recovery if not provided with additional sedation. Reversal of the zolazepam in animals with a benzodiazepine antagonist can lead to an anxious recovery if sufficient plasma levels of tiletamine are still present.

Adverse Effects/ Contraindications

CNS disease: Ketamine/ tiletamine should not be used in animals with elevated intracranial pressure.

Pain from injection: Ketamine is a hydrochloride salt, which must be kept at a low pH to maintain solubility. The pH of ketamine is 3.5 which can cause some irritation and pain during IM injections (Hanna et al., 1988).

Cardiovascular disease: Ketamine/ tiletamine should be used with caution in patients with coronary artery disease, uncontrolled arterial hypertension, cardiomyopathy, or heart failure.

Emergence delirium: Caution should be used in patients where emergence delirium could be injurious to the patient or to medical personnel (e.g., horse). Emergence delirium can be prevented or attenuated by coadministration of sedatives or tranquilizers. In general, dissociative anesthetics should not be administered as sole agents.

Ocular disease: Ketamine/ tiletamine should not be used animals with elevated intraocular pressure or with an open globe injury due to the rise in intraocular pressure following administration.

Increased sympathetic tone: Ketamine/ tiletamine should be avoided in patients with high sympathetic tone such as hyperthyroidism or pheochromocytoma since administration of these drugs can increase norepinephrine levels and exacerbate the side effects of these diseases.

Urethral obstruction or renal insufficiency: Caution should be used in patients where excretion of the active metabolites may be decreased, such as cats with urethral obstruction or anuric renal failure.

Table 12.5 Dose of ketamine and tiletamine in various species

Species	Dosage of ketamine (mg/kg)[a]	Dosage of Telazol® (mg/kg) [b]
Dog	2–5 IV, 5–10 IM	3–6 IM
Cat	5–10 IV, 5–10 IM 10–20 PO 25 rectally	4–7 IM
Cattle	2–4 IV	2–4 IV
Camelid	2–4 IV	0.5–2 IV, 2–4 IM
Ferret	2–5 IV, 5–10 IM	
Horses	2 IV	1–3 IV (following α_2-agonist)
Rat	40–80 IM	30–60
Pigs	10–30 IM	6 IM (following α_2-agonist) 0.01–0.02 ml/kg as TKX[d]
Rabbits	20–50 IM	30–60 IM[c]
Primates	2–4 IV, 10–15 IM	3 IM

[a] Following sedation with benzodiazepine, α_2-agonist or phenothiazine.
[b] Dose for Telazol is for combination of drugs at 100 mg/ml.
[c] Telazol® should not be used in New Zealand White Rabbits.
[d] TKX = 500 mg Telazol® (as powder) + 250 mg ketamine + 250 mg xylazine.

Dose

Dose ranges for ketamine and tiletamine in various species are listed in Table 12.5.

The volume per kg of body weight of Telazol is less than an equipotent amount of ketamine. This makes it attractive for work with zoo/ wildlife animals where the volume may be important (e.g., darts or syringe poles).

Species Differences

Horse: Horses should not be administered dissociative anesthetics as single agents due to the lack of muscle relaxation and recovery delirium. Telazol in particular can be associated with rough anesthetic recoveries and should be balanced with the use of additional sedatives or tranquilizers, before induction and before recovery.

Tigers: Great controversy occurs over the use of Telazol in the large cats and in tigers specifically. Although there is little published evidence, many anesthesiologists and zoo medicine specialists caution against the use of Telazol in the large cats (Tilson, 1994; Muir et al., 2000; Morris, 2001). Reported complications have ranged from slow ataxic recoveries to delayed (2–4 days after anesthesia) neurological dysfunction, including hind limb weakness, drowsiness, hyperreflexia, hyperresponsive behavior, twitches, seizures, and death (Tilson, 1994; Klein, 2007; Lewis, 2007).

Rabbits: At clinical doses, tiletamine (the dissociative part of Telazol) causes lethal renal tubular necrosis in New Zealand White rabbits (Doerning et al., 1992). Other rabbit species appear unaffected.

Pigs: A combination known as TKX (Telazol, ketamine, xylazine) is commonly used to anesthetize pigs. The mixture is composed of 500 mg (as powder) Telazol, 250 mg ketamine, and 250 mg xylazine. Due to slow zolazepam metabolism, pigs can have prolonged recoveries after TKX.

Use of ketamine in animals susceptible to malignant hyperthermia has been controversial; however, ketamine did not induce malignant hyperthermia in 76 susceptible pigs (Dershwitz et al., 1989).

Drug Interactions

The concurrent administration of other CNS depressants can increase respiratory and cardiovascular depression. Chloramphenicol can prolong recovery times from ketamine (Amouzadeh et al., 1989) as well as from Telazol.

Overdosage/Acute Toxicity

Ketamine has a large therapeutic index; therefore, treatment of an overdose is primarily supportive care and addressing side effect such as respiratory or cardiovascular depression, muscle rigidity, emergence delirium, or adrenergic stimulation. There is no direct antagonist for ketamine or tiletamine. Other sedatives and muscle relaxants are used to manage the problems from an overdose until the drug is cleared.

Regulatory Information

Withdrawal time: Ketamine extra label use: at least 3 days for meat and 48 hours for milk.

Controlled drug schedule:

Ketamine is a Schedule III Controlled Drug.
Telazol is a Schedule III Controlled Drug.

Drug Availability

FDA Veterinary Approved Products

Ketamine is approved by the FDA for IM use in the cat and nonhuman primates: KetaFlo, Abbot Laboratories 100 mg/ml.

Telazol is approved by the FDA for IM use in dogs and cats (restricted during pregnancy): Telazol, Fort Dodge Animal Health, Division of Wyeth, 50 mg/ml Tiletamine and 50 mg/ml zolazepam/ ml of solution.

Human Approved Products

Ketalar, Parkedale Pharmaceuticals, 10 mg/ml, 50 mg/ml, 100 mg/ml.

Ketamine Hydrochloride, Hospira, 50 mg/ml, 100 mg/ml.

Etomidate

History

Etomidate was first introduced into human anesthesia practice in 1972. At the time it was considered close to the "ideal anesthetic" because of its ability to maintain hemodynamic stability, minimally depress the respiratory centers, and by having a large therapeutic index (i.e., the lethal dose/ effective dose). However, the ideal anesthetic has yet to be found and etomidate too has untoward side effects.

Classification

Etomidate is a nonscheduled, nonbarbiturate compound suitable for rapid intravenous anesthetic induction.

Chemistry: Etomidate is an ethyl ester of a carboxylated imidazole compound with a chemical formula of *R*-(+)-pentylethyl-1*H*-imidazole-5 carboxylate sulfate (Figure 12.4). The imidazole component allows for differing solubility at different pH concentrations. At a low (acidic) pH, etomidate is water soluble, but becomes lipid soluble at physiological pH. There are two isomers but only the (R)(+) isomer has anesthetic properties.

How supplied: Etomidate is poorly water soluble but highly soluble in alcohol, thus is supplied as a 2 mg/ml solution in 35% propylene glycol at a pH of 6.0. When

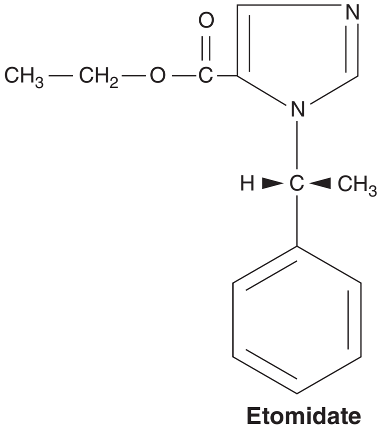


Figure 12.4 Etomidate.

supplied in propylene glycol, etomidate has an osmolarity of ~4620 osmole.

Mechanism of Action

Etomidate is an agonist at the γ-aminobutyric acid (GABA) receptor ($GABA_{Aß3}$) producing hypnosis and CNS depression by enhancing the effects of the inhibitory neurotransmitter GABA (O'Meara et al., 2004). Binding of GABA to the receptors increases chloride binding, resulting in hyperpolarization of the post synaptic neuron and a decrease in neurotransmission.

Indications

Etomidate is indicated for the rapid intravenous induction of anesthesia, particularly in patients with cardiovascular instability.

Physiological Effects

CNS effects: Etomidate is considered cerebral friendly because it results in decreased ICP, decreased CBF, and decreased cerebral metabolic rate for O_2 ($CMRo_2$). Since the MAP is essentially unchanged, this results in a favorable metabolic state for the CNS; the cerebral perfusion pressure is increased as is the cerebral oxygen supply : demand ratio. The EEG quiets (similar to the barbiturates), but there can be increased EEG activity at epileptogenic foci. Grand mal seizure have been reported, and some anesthetists argue against its use in patients with a known seizure history (Bergen and Smith, 1997).

Due to similar mechanisms, the intraocular pressure is also decreased. Intraocular pressure can decrease by up to 60% but the effect lasts for only for ~5 minutes.

Cardiovascular system: Etomidate is unique amongst most intravenous general anesthetics in that it causes minimal hemodynamic changes and thus maintains cardiovascular function. At routine induction doses, etomidate causes minimal changes in stroke volume (SV), MAP, cardiac index (CI), pulmonary artery pressure (PAP), pulmonary arterial wedge pressure (PAWP), central venous pressure (CVP), or systemic vascular resistance (SVR) (De Hert et al., 1990). In addition to causing minimal CV derangements, the baroreceptor and sympathetic nervous system reflexes remain intact, which also contributes to hemodynamic stability. Etomidate is not arrhythmogenic, does not sensitize the heart to catecholamine, and it does not cause histamine release. It is the most commonly used induction agent in dogs having pacemaker implantation (Sanchis-Mora et al., 2014).

Respiratory: Etomidate can cause respiratory depression. Induction doses can result in brief periods of apnea, but generally arterial carbon dioxide partial pressure ($Paco_2$) is only slightly increased if affected at all. Arterial oxygen (Pao_2) is generally unaffected. However, when etomidate is administered with other drugs that can affect CNS function, more respiratory depression can be seen. Since etomidate has limited effect on muscle relaxation, airway reflexes are generally maintained. Intubation, however, is highly recommended.

Endocrine system: In the early 1980s it was noted that there was increased morbidity and mortality in patients sedated long term (e.g., patients on ventilators) with etomidate due to a decreased cortisol production (Wagner et al., 1984). Etomidate causes a reversible inhibition of the enzyme 11-beta-hydroxylase (Figure 12.5), which is an integral part of the pathway that converts cholesterol to glucocorticoids and mineralocorticoids. The resultant decrease in cortisol, corticosterone, and aldosterone production raises concern for patients' ability to respond to stress. Following induction with etomidate, suppression of the adrenal–cortical axis is depressed for up to 6 hours in dogs and 3 hours in cats (Dodam et al., 1990; Moon, 1997). Single induction doses do not cause clinical problems associated with steroid production, but care should be taken with patients with preexisting adrenal–cortical diseases (e.g., hypoadrenocorticism), or who might have relative hypoadrenocorticism, or adrenal depression from chronic disease. Furthermore, extended periods of use (e.g., constant rate infusions) are not recommended due to suppression of cortisol production (as well as the hyperosmolarity of the solution).

Muscular effects: Etomidate does not provide significant muscle relaxation. Muscle rigidity and myoclonus can be seen at, and following, induction but is not associated with seizure-like EEG activity. The use of muscle relaxants (e.g., benzodiazepines) can minimize the incidence and intensity of the muscle movements (Doenicke et al., 1999) and is routinely recommended. Etomidate can potentiate the effects of nondepolarizing neuromuscular blockers.

Analgesia: Etomidate does not provide any analgesia. If patients are anesthetized to facilitate surgery or painful procedures, analgesia must be provided separately with other agents (e.g., opioids, α_2-agonist).

Pharmacokinetics

Following intravenous administration, etomidate is rapidly distributed into the CNS to produce anesthesia. Etomidate is 75% bound to plasma proteins. Three compartment pharmacokinetic models have been used

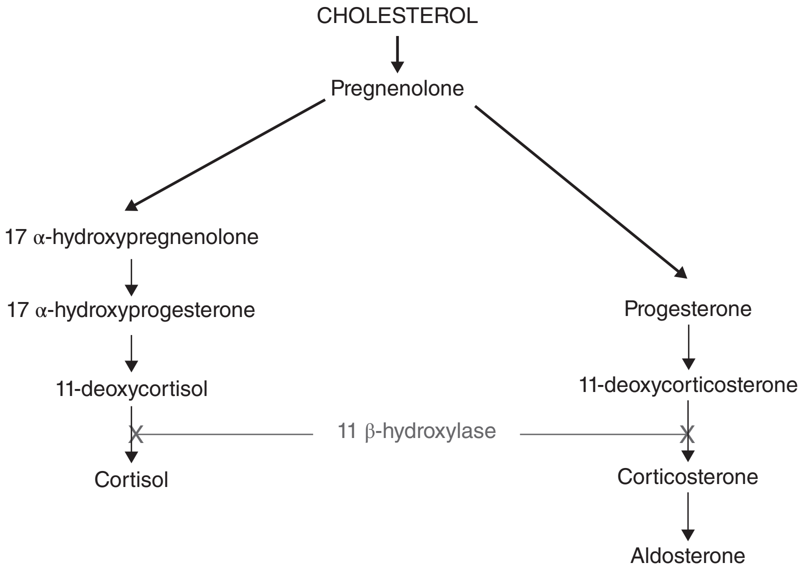


Figure 12.5 Pathway for cortisol and aldosterone production affected by action of etomidate.

to describe etomidate disposition in animals. It has a short redistribution half-life (29 minutes in humans; 22 minutes in cats) and a large steady-state volume of distribution (2.5–4.5 l/kg in humans; 4.9 l/kg in cats). The elimination half-life varies from 2.9 to 5.3 hours in humans and is 2.9 hours in cats (Wertz et al., 1990). Redistribution of etomidate into body tissues is responsible for awakening and recovery time from anesthesia is similar to thiopental, but slower than propofol.

Metabolism

In humans, etomidate is 98% metabolized by liver (2% excreted unchanged in urine) by hydrolysis or glucuronidation via plasma esterases and hepatic microsomal enzymes. Metabolites are inactive and excreted in the urine (85%) and bile and feces (15%). Etomidate should be used cautiously in patients with liver disease as metabolism can be prolonged.

Adverse Effects/ Contraindications

Adverse effects of adrenocortical suppression and myoclonus have been discussed above. Other adverse effects of etomidate arise from the propylene glycol vehicle. The intravenous injection of propylene glycol can be painful. Pain can be minimized by administering etomidate through a running IV line or by injecting lidocaine (dog) through the catheter before induction. Furthermore propylene glycol causes the solution to be hyperosmotic (~4620 Osm) compared to plasma (~300 Osm), and thus etomidate has been associated with intravascular hemolysis (Moon, 1994). Clinically relevant hemolysis has been reported following prolonged administration (i.e., constant rate infusions) in dogs (Ko, 1993; Moon, 1994). Etomidate has been prepared in a lipid vehicle (Etomidate-lipuro, Braun, Germany) instead of propylene glycol, but it is not available in the United States.

Dose (Box 12.2)

Etomidate has wide safety margin, due to a large therapeutic index of 16 (i.e., lethal dose is 16 times the hypnotic dose) compared to the therapeutic indexes for propofol and thiopental (3 and 5, respectively). Etomidate should be titrated intravenously “to effect”. The wide dose range reflects the additive and sometimes synergistic effects of many premedications (e.g., opioids, benzodiazepines, phenothiazines). As discussed previously, etomidate should be administered following an adequate premedication. Duration of anesthesia is directly related to dose. Constant rate infusions are not recommended due to the potential for adrenocortical suppression and hemolysis (see Section Adverse Effects/ Contraindications).

Species Differences

Cats: Due to the extensive liver metabolism of etomidate and fragility of feline red blood cells, cats may be more likely to have clinical hemolysis following etomidate administration.

Box 12.2 Dose for etomidate in animals

Dogs: 0.5–4.0 mg/kg IV
Cats: 0.5–4.0 mg/kg IV
Pigs: 2–4 mg/kg IV
Mice: 11 mg/ kg IV; 24 mg/kg IP

Dogs: Clinical hemolysis has been seen following constant rate infusions.

Swine: Etomidate did not trigger malignant hyperthermia in susceptible pigs (Suresh and Nelson, 1985).

Drug Interactions

Concurrent use of other drugs that depress the CNS (e.g., barbiturates, opioids, general anesthetics, etc.) can potentiate the effects of etomidate.

Overdosage/Acute Toxicity

Acute overdoses would be expected to cause enhanced pharmacological effects of the drug. Treatment would be supportive (i.e., mechanical ventilation), until the effects of the medication are diminished.

Storage/Stability/Compatibility

Unless otherwise labeled, store etomidate injection at room temperature and protect from light.

Regulatory Information

Controlled drug status: Etomidate is a nonscheduled drug.

Drug Availability

FDA veterinary approved products

None

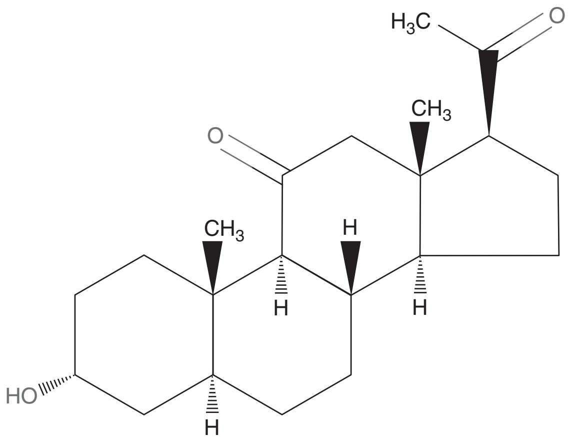
Human approved products

Etomidate Injection 2 mg/ml in 10 ml, 20 ml ampules, single use vials and 20 ml Abboject Syringes; Amidate® (Hospira); generic (Bedford); (Rx).

Neurosteroid Anesthetics

History

Synthetic neuroactive (nonhormonal) steroids have been intermittently popular in human and veterinary anesthesia since the 1970s. Althesin and Saffan® were mixtures of two neurosteroids, alfaxalone and alphadolone, which were used in Canada and the United Kingdom. Although these drugs produced anesthesia, significant side effects due to the solubilizing agent (chromophore) limited their popularity. Both products have been removed from the market. Specific information regarding these products can be found in previous editions of this book.



Alfaxalone

Figure 12.6 Alfaxalone.

Alfaxalone as a single agent was reintroduced into veterinary practice in Australia and United Kingdom in 2000 and has become a popular anesthetic in veterinary medicine. It was approved by the FDA for use in animals in 2014. There is no equivalent human product available.

Classification

Chemistry: Alfaxalone (3-alpha-hydroxy-5-alpha-pregnane-11,20-dione) is a steroid compound (Figure 12.6).

How supplied: Alfaxan®: Each ml contains 10 mg alfaxalone (1%) in 2-hydroxypropyl-beta cyclodextrin. Alfaxalone is highly lipophilic and therefore difficult to solubilize in water-based solutions. Alfaxan-CD inserts the alfaxalone molecule within a cyclodextrin (sugar) structure (Figure 12.7) to allow solubilization in water. Once administered, the dextran is metabolized and the free alfaxalone is bioavailable. The solution has a pH of 6.5–7.0 and has no preservative. The manufacturer recommends that unused product be stored in sterile syringes and unused drug discarded 6 hours after the bottle is opened.

Mechanism of Action

Neuroactive steroids enhance the effects γ-aminobutyric acid (GABA) at the $GABA_A$ receptor. Enhanced activation of the GABA receptors increases transmembrane chloride conductance resulting in hyperpolarization of the postsynaptic cell membrane, which causes inhibition of the postsynaptic neuron. This results in inhibition of pathway activation controlling arousal and awareness.

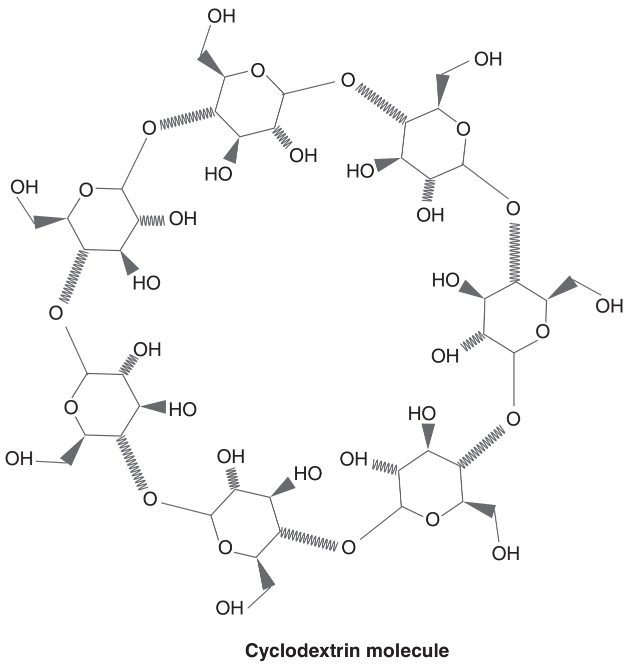


Figure 12.7 Cyclodextrin molecule used for complexing alfaxalone.

Indications

Alfaxalone is indicated for the intravenous induction of general anesthesia in dogs and cats.

Physiological/ Pharmacodynamic Effects

CNS effects: Neurosteroids produce unconsciousness and anesthesia along with a dose-dependent decrease in EEG activity (Zattoni et al., 1980). They produce a favorable neurological environment by decreasing cerebral blood flow, intracranial pressure, and cerebral metabolic demands (Rasmussen et al., 1978).

Cardiovascular system: Alfaxalone decreases arterial blood pressure and systemic vascular resistance (Rodríguez et al., 2012). Although the blood pressure changes are mild and generally not clinically relevant (Muir et al., 2008), the blood pressure in cats may be more affected (Muir et al., 2009). The decrease in blood pressure is associated with an increased heart rate and increased cardiac index (Rodríguez et al., 2012). Administration of alfaxalone with general anesthetic gases (e.g., isoflurane) can result in clinically relevant hypotension.

Respiratory: Alfaxalone is associated with a dose-dependent respiratory depression that may progress to apnea (Muir et al., 2008). All patients administered alfaxalone must be monitored for the need for endotracheal intubation and ventilatory support.

Muscular effects: Alfaxalone provides good muscle relaxation when used alone and is associated with good quality inductions and recoveries (Rodríguez et al., 2012).

Cesarean section: Anesthesia with alfaxalone is associated with good puppy viability following cesarean section and is associated with better APGAR scores in puppies compared with dogs anesthetized with propofol (Doebeli et al., 2013).

Miscellaneous: Perivascular injection does not cause tissue damage nor does it appear to be painful. Alfaxalone does not bind glucocorticoid, mineralocorticoid, or sex hormone receptors. There is no evidence that it clinically interferes with mineralocorticoid or glucocorticoid production. Alfaxalone has been successfully used in dogs and cats less than 12 weeks of age (O'Hagan et al., 2012), but has not been evaluated in kittens less than 4 weeks old or puppies less than 10 weeks old.

Allergic reactions: Earlier versions of the neurosteroid formulation were associated with allergic reactions ranging from erythema and edema to life-threatening anaphylaxis associated with the vehicle, which contained Cremophor. Further information regarding these reactions may be found in older editions of this book. Complexing with cyclodextrin to maintain solubility for injection has apparently solved this problem with adverse reactions, and currently marketed alfaxalone in a cyclodextrin molecule does not appear to cause allergic reactions in dogs or cats (Ferré et al., 2006).

Analgesia: Neurosteroids do not produce analgesia mediated through GABA_A receptors (Nadeson and Goodchild, 2000).

Pharmacokinetics

See Box 12.3 (Goodwin et al., 2011).

Box 12.3 Alfaxalone pharmacokinetics

Species	Half-life (min)	Volume of distribution (l/kg)	Dose IV^{a} (mg/kg)
Dogs	34	2.0	1–3
Cats	43	1.3	2–5
Horses	33.4	1.6	

[a] To effect.

Metabolism

Alfaxan is metabolized in the liver and eliminated in the bile and urine (Jurox Limited, 2014), as has been reported for other neurosteroids (Strunin et al., 1977).

Adverse Effects/ Contraindications

As with all CNS depressants, patients should be individually assessed for risks associated with anesthesia, myocardial depression, and ventilatory depression. Combining of CNS depressants can result in synergistic effects and make detrimental side effects more likely (e.g., hypoventilation).

Administration

The dose of alfaxalone (Box 12.3) should be titrated for each patient. It should be slowly administered IV until the patient is anesthetized ("to effect"). Administration of other CNS depressants as premedications will reduce the total amount of alfaxalone needed.

Although considered an off label administration route, intramuscular and subcutaneous (Heit et al., 2004) injection of alfaxalone will produced deep sedation. However, recovery following IM injection of alfaxalone is associated with excitability in cats even with adjunct sedation (Grubb et al., 2013).

Species Differences

Dog: Alfaxalone is a reasonable alternative to propofol in Greyhound dogs. Pharmacokinetics in Greyhound dogs were no different than Beagle dogs (Pasloske et al., 2009).

Horses: Alfaxalone has been used to produce general anesthesia in horses following premedication with acepromazine, xylazine, and guaifenesin. Induction scores with this protocol were good and the recoveries were considered satisfactory (Goodwin et al., 2011).

Alpaca: Alfaxalone produced general anesthesia with good inductions in alpaca; however, its use as a solo anesthetic agent was associated with poor anesthetic recoveries (Del Álamo et al., 2014).

Sheep: Alfaxalone produced anesthesia at 2 mg/kg IV with minimal cardiovascular effects, no reported apnea, and good recoveries (Andaluz et al., 2012).

Rabbits: Alfaxalone at 2–3 mg/kg IV produced anesthesia in rabbits, but was associated with apnea of ~45 seconds (Grint et al., 2008).

Overdosage/Acute Toxicity

Alfaxalone has a large therapeutic index and a short duration of action (Ferré et al., 2006). Alfaxalone has been administered at 10 times and five times the recommended dose in dogs and cats, respectively, with only the need for ventilatory support for full recovery (Muir et al., 2008; Muir et al., 2009). The half-life is short (Box 12.3), therefore adverse effects should dissipate quickly because of rapid elimination. Treatment of an overdose should focus on supportive care, particularly of the respiratory system, until recovery occurs.

Regulatory Information

Controlled drug status: Alfaxalone is a schedule IV drug.

Withdrawal information: No information available.

Drug Availability

FDA Veterinary Approved Products

Alfaxan (alfaxalone, 10 mg/ml): Jurox Inc. American Century Tower II, Kansas City, MO.

Human Products

None available

Miscellaneous Anesthetics

Alpha-Chloralose

History

Alpha-chloralose is a compound from the mixture of glucose and chloraldehyde. It has been used for sedation and immobilization of animals since the turn of the 20th century. It currently is not approved for use by the FDA; however, under FDA regulation it can be used in a variety of species. It is most commonly used in laboratory animal medicine, but has also been used to sedate wild (nuisance) birds to allow aesthetic removal from unwanted places (e.g., parks, schools, etc.)(O'Hare et al., 2007).

Chemistry

Chloralose, 1,2-O-(2,2,2-trichloroethylidene)-alpha-D-glucofuranose (Figure 12.8), is prepared by condensing anhydrous glucose with chloraldehyde (chloral) in the presence of sulfuric acid. A mixture of α-chloralose and β-chloralose is formed, of which the α-chloralose form is the active form. Chloralose is difficult to dissolve in an aqueous medium without simultaneous heating, but the solution should not be boiled.

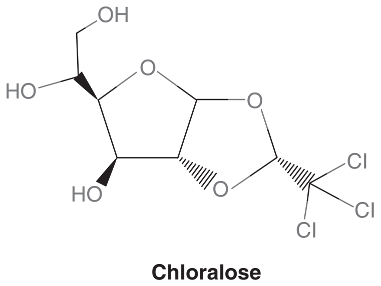


Figure 12.8 Chloralose.

Box 12.4 Chloralose doses in various species (administered as a single dose)

Dog	40–110 mg/kg IV
Cat	40–80 mg/kg IV
Sheep	45–55 mg/kg IV
Pig	55–86 mg/kg IV
Waterfowl	30 mg/kg PO
Pigeons	180 mg/kg PO
Other birds	15–50 mg/kg PO

How supplied: Alpha-chloralose usually is prepared and administered intravenously in 1% concentration. It has also been used orally in a variety of wild bird species (turkeys, pigeons, geese, etc.).

Mechanism of Action

The mechanism of action has not been identified. An active metabolite of α-chloralose, trichloroethanol, is the same as for the sedative, chloral hydrate. It is likely that both have interaction at the GABA receptor (see section on chloral hydrate later in this chapter)(Garrett and Gan, 1998).

Indication

Alpha-chloralose is best used as a sedative or hypnotic as it is not a complete general anesthetic. α-chloralose is restricted to terminal studies in laboratory animals in which recovery from anesthesia is not necessary. It is used often used in physiological experimentation because it purportedly does not interfere with respiratory and cardiac reflexes, for example, baroceptor and chemoceptor activities. It can also be used to sedate wild birds to allow for capture.

Physiology

Chloralose induces prolonged hypnosis, lasting up to 8–10 hours. It is metabolized to chloraldehyde or chloral, which is mainly transformed into trichloroethanol (a metabolite of chloral hydrate). Hypnosis and anesthesia produced by chloral hydrate and chloralose are quite similar because of the formation of same metabolite, trichloroethanol.

Alpha-chloralose was considered an ideal drug for sedation/anesthesia of research animals due to its purported ability to provide CNS depression while not depressing baroreceptor reflexes, vasomotor centers, or spinal reflexes. However, this is been shown to be untrue in rats, lambs, and rabbits (Fish and Meyer, 2008). Additionally, spinal reflex activity may increase to the degree that muscle activity ("convulsions") similar to those of strychnine poisoning develop in the dog and cat (Lees, 1972). Paddling movements and muscle fasciculations have been seen in pigs, sheep, and other species. Surgical anesthesia cannot be reached by administration of chloralose alone.

The degree of CNS depression that is produced by the drug is unknown. When α-chloralose overdose has occurred in people, they show marked CNS depression, with depressed to flat EEG waves (Manzo et al., 1979). Furthermore, people showed respiratory depression, muscle fasciculations, and seizure-like activity, similar to what had been reported in the dog (Lees, 1972; Manzo et al., 1979). Its effectiveness as an anesthetic varies among species, and is least effective in the dog. It is not recommended for survival procedures because of rough induction, prolonged recovery, and seizure-like activity (Silverman and Muir, 1993).

There is no evidence that α-chloralose provides analgesia and should not be used as single agents for surgical interventions (Silverman and Muir, 1993).

Intraperitoneal injections produce pain and inflammation and are not recommended.

Dose

See Box 12.4

Regulatory Information

Controlled drug status: Nonscheduled drug.

Drug Availability

There are no FDA Veterinary Approved nor Human Approved products.

Urethane

History

Urethane was a popular anesthetic for laboratory animals because it produces a long duration from a single administration and minimal physiological effects. It is no longer recommended because it is carcinogenic; however, it is still used for laboratory animal research (Lucking et al., 2014).

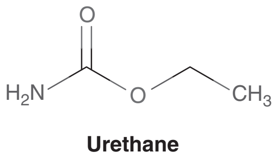


Figure 12.9 Urethane.

Chemistry

Urethane (ethyl carbamate) is the ethyl ester of carbamic acid (Figure 12.9). It is chemically related to urea and is readily soluble in water and alcohol.

Mechanism of Action

The mechanism of action of urethane has been poorly studied. It has been shown in rats that urethane selectively alters potassium currents to depress neuronal excitability (Sceniak and MacIver, 2005). This is a unique mechanism for an anesthetic.

Physiological Effects

Urethane produces long-lasting unconsciousness of 8–10 hours duration after a single dose. Spinal reflexes, neural transmission, and cardiopulmonary function are minimally affected. Unlike chloralose, urethane appears to induce analgesia and muscle relaxation. Urethane has been administered by most routes, including topically to frogs.

Metabolism

Urethane is metabolized slowly by cytochrome P450 into vinyl carbamate, vinyl carbonate epoxide, and eventually into CO_2 and NH_3. The rate of elimination is slow is associated with long difficult recoveries.

Carcinogenicity

Urethane is on the National Institute of Health's list of drugs that are "reasonably anticipated to be a human carcinogen" (USPHS, 2014). Exposure can be by inhalation, ingestion, or dermal contact. Strict precautions should be taken to prevent exposure to personnel.

Dose

Urethane is not recommended for used due to its potential as a carcinogen and because there are alternative anesthetics available. Rodents: 1 g/kg IV, 1–2 g/kg IP.

Regulatory Information

Controlled drug status: Nonscheduled drug

Drug Availability

There are no FDA Veterinary Approved nor Human Approved Products

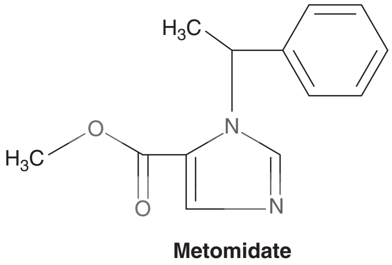


Figure 12.10 Metomidate.

Metomidate

History

Metomidate is a nonbarbiturate imidazole anesthetic that is in the same family as etomidate. It had been used as an anesthetic in mammals and birds, but currently is primarily used as a fish anesthetic. Because of a similar spelling, it has erroneously been confused with medetomidine (see Chapter 14), but they are very different agents.

Chemistry

Metomidate (methyl 1-(alpha-methylbenzyl)imidazole-5-carboxylate) is an imidazole compound (Figure 12.10).

How Supplied

Metomidate is white powder soluble in water and ethanol. It needs to be reconstituted in aqueous solutions, which are acidic.

Mechanism of Action

Imidazole anesthetics are γ-aminobutyric acid (GABA) receptor agonists that produce anesthesia and amnesia (Stoelting and Hillier, 2012).

Indications

Metomidate is primarily used to produce anesthesia in finfish.

Pharmacokinetics

Metomidate was rapidly absorbed over the gills and produced anesthesia within 1 minute at exposure to 9 mg/l (Hansen et al., 2003). Oral bioavailability in turbot fish was 100% (Hansen et al., 2003).

Physiology

Metomidate produces hypnosis, anesthesia, and muscle relaxation. Cardiovascular function is generally preserved, but respiratory depression and apnea can occur. They are poor muscle relaxants and provide no analgesia.

Imidazoles (metomidate and etomidate) have been credited with providing complete anesthesia and decreasing the stress response. Unfortunately, the stress-free aspects attributed to these drugs are a complete misnomer. Metomidate blocks the 11-beta hydroxylation of cortisol both in mammals and in fishes. This seriously perturbs the complex positive and negative feedback pathways for control of the neuroendocrine cascade. It is therefore likely that animals are experiencing the same stress, but are not able to respond to it by producing cortisol. Researchers should be cautious in interpreting stress response in animals exposed to imidazole anesthetics.

Fish exposed to metomidate can have dark skin discoloration after exposure. Because cortisol inhibits the release of adrenocorticotrophic hormone (ACTH) and ACTH stimulates melanocyte stimulating hormone, it is hypothesized that the decrease in cortisol production that occurs with the use of these drugs results in an increase in melanocyte stimulating hormone (Harms and Bakal, 1994).

Doses

Aquacalm^{TM}: Immersion agent for fish. The dose for sedation is 0.1–1.0 mg/l, and the dose for anesthesia is 1.0–10 mg/l.

Species Differences

Frogs: Leopard frogs exposed to metomidate by immersion in 30 mg/l became sedated, but only 27% became anesthetized (Doss et al., 2014). Furthermore, recovery times were extremely prolonged (up to 5 hours)(Doss et al., 2014).

Regulatory Information

Metomidate is a noncontrolled drug.

Drug Availability

FDA veterinary approved products: Aquacalm^{TM} is legally marketed as an FDA indexed product under MIF 900-002 (Western Chemical Inc., 2009). It is approved for ornamental finfish only. Each gram of Aquacalm^{TN} contains 1 gram of metomidate hydrochloride. Available in 1, 5, 10, 50-gram bottles

Human approved products: None

Tribromoethanol

History

Tribromoethanol is an injectable anesthetic agent used in mice. It was manufactured as Avertin® but this product is no longer available. Investigators who wish to use tribromoethanol as an anesthetic must make their own solutions.

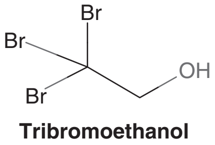


Figure 12.11 Tribromoethanol.

Chemistry

2,2,2-Tribromoethanol (Figure 12.11).

Mechanism of Action

Tribromoethanol interacts with the GABA$_A$ and glycine receptors (Krasowski and Harrison, 2000). It may also have effects at dopamine, serotonin, and opioid receptors due to its ethanol properties (Fish and Meyer, 2008).

Physiology

Tribromoethanol produces generalized CNS depression including the cardiovascular and respiratory centers. Duration of affect is species, strain, and route of administration dependant. Dogs and cats administered tribromoethanol rectally showed sedation for ~1 hour and 24 hours, respectively. Different strains of mice have significantly different dosing requirements (125 vs. 500 mg/kg IP) (Fish and Meyer, 2008).

It is undergoes hepatic conjugation and the glucuronide is excreted in the urine in rodents (Green et al., 1998). Tribromoethanol produces repeatable anesthesia, but sleep times can be variable in young or sick animals (Meyer and Fish, 2005).

Adverse Effects

Tribromoethanol is an irritant and produces inflammation and peritonitis when administered intraperitoneally, especially at high doses, high concentrations, or with repeated use (Meyer and Fish, 2005). Anesthesia with tribromoethanol can result in ileus, abdominal adhesions, increased generalized morbidity, as well as increased mortality (Meyer and Fish, 2005). It is recommended only for acute terminal studies when administered intraperitoneally (Fish and Meyer, 2008).

Tribromoethanol has been associated with impaired fertility (Meyer and Fish, 2005). Tribromoethanol degrades in the presence of heat or light to produce toxic byproducts. Degraded solutions can be both nephrotoxic and hepatotoxic. Administration of degraded tribromoethanol solutions has been associated with death, often 24 hours after surgery.

Dose

Due to the increased morbidity and mortality following anesthesia, and repeated anesthetics, tribromoethanol is recommended only for terminal studies (Fish and Meyer,

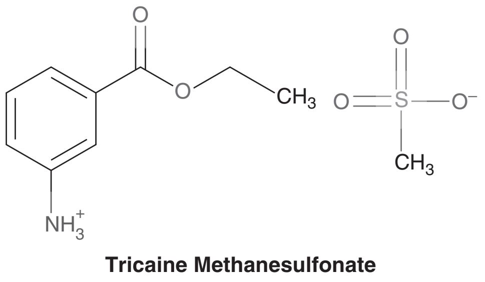


Figure 12.12 Tricaine methanesulfonate.

2008). The dose administered to mice is 150–500 mg/kg IP

Regulatory Information

Nonscheduled drug

Drug Availability

There are no FDA veterinary approved nor human approved products. When used, drug must be reconstituted from chemical powder.

Tricaine Methanesulfonate (MS-222)

History

Tricaine is the only anesthetic approved by the United States Food and Drug Administration for fish intended as food and in the USA is the most commonly used agent for the anesthesia of fish.

Chemistry

Tricaine (3-aminobenzoic acid ethyl ester methanesulfonate; Figure 12.12) is a white crystalline power that has a water solubility of 1.25 g/ml at 20°C. A stock solution of 10 g/l can be stored in an airtight container at room temperature. The solution should be stored in darkened containers as sunlight will cause the solution to turn brown. The addition of tricaine to water at relevant doses will cause the water to become acidic. At 100 mg/l the pH of the solution can be as low 5. It is therefore recommended to buffer the solution with sodium bicarbonate to a pH between 7.0 and 7.5.

Mechanism of Action

Tricaine produces generalized CNS depression but its direct mechanism of action is unknown. Tricaine is a water-soluble chemical structurally related to benzocaine, a local anesthetic. Local anesthetic drugs works by blocking conduction of sodium channels (see Chapter 15). It is reasonable to question if the lack of movement seen in fish anesthetized with tricaine is at least partially associated with blocked conduction of muscle as opposed to true CNS depression. There is evidence of impacts on nervous tissues as tricaine does decrease neural conduction in toadfish (Palmer and Mensinger, 2004). The structurally related drug, lidocaine, has also been shown to be analgesic and to function as a CNS depressant when given centrally in domestic mammals (Doherty and Frazier, 1998; Valverde et al., 2004). It is possible that tricaine may act similarly in fishes.

Physiology

Tricaine is rapidly absorbed via gill diffusion and provides a fairly rapid induction and recovery. The early stages can show some excitatory effects similar to those seen in terrestrial animals as they pass through an excitatory state (stage II anesthesia) before becoming immobile. Once immobile, tricaine is able to completely abolish muscle movement.

Physiological effects of tricaine include a decrease in heart rate, cardiac contractility, and dorsal aortic pressure (Hill et al., 2002). Anesthesia with tricaine also increased cortisol, glucose, hematocrit, and lactate (Cho and Heath, 2000).

Adverse Effects

Tricaine has a high therapeutic index and morbidity and mortality are rare, even with fish anesthetized for hours (Harms et al., 2005), provided physiological needs are met (e.g., water flow over gills).

Dose

The dose typically used in fish for anesthesia is 25–150 mg/l for induction of anesthesia, and 400–500 mg/l to produce euthanasia.

Regulatory Information

Controlled drug status: Nonscheduled drug.

Withdrawal information: Withdrawal time 21 days for fish harvested for food.

Drug Availability

FDA veterinary approved products:

Finquel, Argent Laboratories, powder for reconstitution
Tricaine-S, Western Chemical, Inc, powder for reconstitution

Human approved products: None

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13

Opioid Analgesic Drugs

Butch KuKanich and Mark G. Papich

Introduction

The opioids include drugs that are true opiates – derivatives of opium – and drugs that are synthetic agents but also affect opiate receptors. Opiates include morphine, codeine, and thebaine isolated from the poppy plant. These drugs differ among one another primarily in potency, but also may have different pharmacokinetic and pharmacodynamic properties. Opioids include all the opiates, as well as synthetic drugs, such as fentanyl, methadone, and meperidine (pethidine) that are not chemically related to morphine but act as opiate receptor agonists. Various types of opiate receptors will be discussed later in the chapter.

The opioids are valuable tools in the veterinarian's armamentarium of drugs. Although most of the drugs in this group are controlled substances because of their potential for abuse by humans, they can have profound effects for treatment of animals (Table 13.1). There are many overlapping areas with other therapeutic uses discussed in other chapters of this book (for example Chapter 46 dealing with gastrointestinal drugs and Chapter 48 dealing with respiratory drugs – antitussives).

An important advantage of opioid analgesic drugs is their high efficacy and remarkable safety. If adverse effects are recognized, their short half-lives in animals usually produce a rapid alleviation of clinical signs. If adverse reactions are severe (e.g., dysphoria or life-threatening respiratory depression) these drugs also have the benefit of reversibility, which is accomplished quickly with administration of an opioid antagonist such as naloxone. This lack of serious adverse effects allows clinicians to gradually escalate doses of opioid agonists in patients as needed for pain or decrease dose if adverse effects occur in highly sensitive animals. Opioid agonists/antagonists may have a ceiling effect limiting the effectiveness of high doses, as discussed later.

Background on Opioids

Opioid Role in Analgesia

A nociceptive stimulus produces an unpleasant sensation. Pain is the coupling of the unpleasant sensation with conscious perception and eliciting an emotional response. There is debate on whether all animals have true emotional responses, and thus whether they can feel pain according to its definition, but there is no debate that nociception is present in animals. Nociceptive receptors are free nerve endings distributed throughout the body, which detect a nociceptive stimulus. Nociceptive stimuli are transmitted from peripheral nerves though the spinal cord to numerous areas within the central nervous system. Mechanical (pressure, fractures, stretching), temperature (hear or cold), and chemical (including endogenous substances such as inflammatory cytokines and changes in tissue pH) stimuli are all types of stimuli that produce pain. For the discussions in this chapter pain and nociception will be used synonymously.

Large strides have been made recently in the recognition and treatment of pain in animals. Numerous pharmacokinetic and pharmacodynamic studies have been performed in order to better gain our understanding of opioids in animals through laboratory and clinical studies. Treatment regimens used in humans cannot be easily extrapolated to animal species treated by veterinarians because of species differences in pain sensation, pharmacokinetic differences, efficacy, and adverse effects of opioids.

History of Opioids

Opioids are considered the prototypical analgesic, antidiarrheal, and antitussive drugs by which other drugs are compared. Opium was first imported into the

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Table 13.1 Therapeutic uses of opioids in animals

Analgesia (antinociception)
Sedation
Calming and euphoria
Immobilization and chemical restraint
Diarrhea, inhibition of gastrointestinal motility
Antitussive
Adjunctive use for general anesthesia
Increased locomotor activity (used illegally in performance horses for this purpose)

United States in 1840, primarily from the country of Turkey. It was widely used to treat a variety of maladies, including pain, diarrhea, and coughing. In the 1870s, because of widespread use after the Civil War, opiate use and addiction was a recognized problem. There are over 20 opiate alkaloids in opium and in the early 19th century, the chemist Sertürner isolated morphine, which he named after *Morpheus*, the Greek god of sleep and dreams. Heroin was also developed in the early 1900s by the same chemists at Bayer who developed aspirin. It was intended to be a safe and nonaddictive analgesic. There have been other attempts – none completely successful – during the next century to develop drugs as effective as the opiates, but without the abuse and addiction potential. The majority of opioids are now controlled by regulatory authorities.

In the United States, the Drug Enforcement Agency (DEA) and Food and Drug Administration (FDA) are the regulatory agencies. (Regulation of drugs is discussed in more detail in Chapter 55. Prescribing controlled drugs is discussed in Chapter 56.) The FDA approves drugs based on efficacy and safety, and drugs with the potential for abuse are scheduled by the DEA on the basis of its addictive potential (Schedule I through V). Schedule I (CI) drugs have no realistic medical benefit (e.g., heroin). Schedule II (CII) drugs can be highly addictive, but have important therapeutic uses. Morphine and most of the pure agonists are listed in Schedule II (Table 13.2). Schedule III (CIII) have an abuse potential, but less than CII drugs. Schedule IV (CIV) drugs have an abuse potential, but less than CIII drugs and Schedule V (CV) drugs have an abuse potential, but less than CIV drugs. Occasionally, a drug's schedule may change. For example, the DEA recently changed hydrocodone combination drugs (e.g., hydrocodone with acetaminophen) from CIII to CII drugs, and tramadol went from being an unscheduled drug to Schedule IV.

In veterinary medicine, the earliest recorded use of opium was in 1815, when it was considered important in the *Materia Medica* (Stalheim, 1990) (See Chapter 1 for a discussion of the *Materia Medica*.) In 1930, morphine was first recommended in a veterinary pharmacology textbook for treatment of horses for colic, cough, and analgesia. Although recommended for treatment of pain and colic in horses at that time, the adverse effects in horses were not recognized. Other drugs used in the early 20th century by veterinarians included combinations of morphine and atropine for pain in dogs and the use of paregoric (tincture of opium) for treatment of flatulence. Early veterinary preparations included paregoric, laudanum (opium), ipecac and opium (Diver's powder), morphine, heroin (0.5 to 2 grains for a horse), codeine, and dionin (ethylmorphine) (Stalheim, 1990).

Veterinary medicine has advanced considerably since those early years and it is common for modern veterinary hospitals to include morphine and other potent derivatives in their pharmacy. Injectable solutions for intermittent administration or constant rate infusion, oral formulations, and transdermal delivery devices are common in most veterinary hospitals used as preanesthetics, anesthetic adjuncts, and to provide analgesia among other uses.

Physiology of Opioids

Three main classes of opiate receptors have been identified: μ, κ, and δ (Table 13.3). Numerous opiate receptor subtypes have also been identified. In some textbooks, a sigma (σ) receptor is listed, but the significance of this receptor is not understood and is not considered a true opiate receptor by many pharmacologists.

Clinically, differences in mu (μ)-opioid effects have been observed in dogs with marked pharmacodynamic variability of effects. A morphine dose that produces desirable and predictable effects in most dogs may produce profound sedation in some individuals and marked dysphoria in others. In the past, these ranges of responses have been suggested to be "sensitivity" differences as the doses and plasma concentrations were within expected ranges. However, recent data suggest opioid receptor subtypes exist, which may contribute to the variability in opioid effects, but are not well characterized in dogs. Methadone combined with morphine and codeine produces a synergistic analgesic effect in mice, but only additive effects with other opioids such as oxymorphone and fentanyl, suggesting different receptor binding for morphine and methadone (Bolan et al., 2002). μ-opioid deficient mice (CXBX strain) are insensitive to morphine, but still respond to some other opioids including methadone and fentanyl, which also suggests μ-opioid subtypes exist (Vaught et al., 1988; Chang et al., 1998). Although only one μ-opioid receptor gene has been identified, multiple μ-opioid splice variants have been documented in rats, which may explain differential agonist effects (Verzillo et al., 2014). There have also been single nucleotide polymorphisms identified in the μ-opioid gene of dogs, which may play a role in opioid-induced dysphoria (Hawley and Wetmore, 2010).

Table 13.2 United States drug scheduling of the opioid narcotics; complete list may be found at: http://www.usdoj.gov/dea/pubs/scheduling.html. Source: United States Drug Enforcement Administration.

Substance	DEA number	Other names and brand names
Schedule I		
Acetyldihydrocodeine	9051	Acetylcodone
Benzylmorphine	9052	
Codeine methylbromide	9070	
Codeine-N-oxide	9053	
Desomorphine	9055	
Dihydromorphine	9145	
Etorphine (except HCl)	9056	
Heroin	9200	Diacetylmorphine, diamorphine
Morphine methylbromide	9305	
Morphine methylsulfonate	9306	
Morphine-N-oxide	9307	
Nicomorphine	9312	Vilan
Normorphine	9313	
Schedule II		
Alfentanil	9737	Alfenta
Carfentanil	9743	Wildnil
Dihydrocodeine	9120	Didrate, Parzone
Diphenoxylate	9170	
Diprenorphine	9058	M50-50
Ethylmorphine	9190	Dionin
Etorphine HCl	9059	M 99
Fentanyl	9801	Innovar, Sublimaze, Duragesic
Hydrocodone	9193	dihydrocodeinone
Hydrocodone and isoquinoline alkaloid 15 mg/du	9805	Dihydrocodeinone, papaverine or noscapine
Hydrocodone combination product 15 mg/du	9806	Tussionex, Tussend, Lortab, Vicodin, Hycodan, Anexsia
Hydromorphone	9150	Dilaudid, dihydromorphinone
Levomethorphan	9210	
Levorphanol	9220	Levo-Dromoran
Meperidine	9230	Demerol, Mepergan, pethidine
Methadone	9250	Dolophine, Methadose, Amidone
Methadone intermediate	9254	Methadone precursor
Morphine	9300	MS Contin, Roxanol, Duramorph, RMS, MSIR
Opium extracts	9610	
Opium fluid extract	9620	
Opium poppy	9650	Papaver somniferum
Opium tincture	9630	Laudanum
Opium, granulated	9640	Granulated opium
Opium, powdered	9639	Powdered Opium
Opium, raw	9600	Raw opium, gum opium
Oxycodone	9143	OxyContin, Percocet, Tylox, Roxicodone, Roxicet,
Oxymorphone	9652	Numorphan
Racemethorphan	9732	
Racemorphan	9733	Dromoran
Remifentanil	9739	Ultiva
Sufentanil	9740	Sufenta
Schedule III		
Buprenorphine	9064	Buprenex, Temgesic
Butabarbital	2100	Butisol, Butibel
Codeine combination product 90 mg/du	9804	Empirin, Fiorinal, Tylenol, ASA or APAP w/codeine
Nalorphine	9400	Nalline
Schedule IV		
Butorphanol	9720	Stadol, Stadol NS, Torbugesic, Torbutrol
Pentazocine	9709	Talwin, Talwin NX, Talacen, Talwin Compound
Schedule V		
Codeine preparations: 200 mg/100 ml or 100 g		Cosanyl, Robitussin A-C, Cheracol, Cerose, Pediacof
Dihydrocodeine preparations: 10 mg/100 ml or 100 g		Cophene-S, various others
Diphenoxylate preparations: 2.5 mg/25 μg atropine sulfate		Lomotil, Logen
Ethylmorphine preparations: 100 mg/100 ml or 100 g		
Opium preparations: 100 mg/100 ml or 100 g		Parepectolin, Kapectolin PG, Kaolin Pectin P.G.

Table 13.3 Opiate receptor types and their associated effects (some textbooks also list the sigma-receptor, but this may not have any significance)

μ Receptor	κ Receptor	δ Receptor
Analgesia (spinal and supraspinal)	Analgesia (spinal and supraspinal)	Analgesia (spinal and supraspinal)
Respiratory depression		
Decrease gastrointestinal motility	Decrease gastrointestinal motility	
Decrease gastrointestinal secretions	Decrease gastrointestinal secretions	
Decrease biliary secretions		
Increase appetite	Increase appetite	Increase appetite
Sedation/excitement (dose and species dependent)	Sedation	
Euphoria		
Antidiuresis	Diuresis (decrease ADH release)	
Decrease urine voiding reflex		
Decrease uterine contractions		
Miosis/mydriasis (species specific)	Miosis/mydriasis (species specific)	
Nausea/vomiting or antiemetic (drug specific)	Nausea/vomiting or antiemetic (drug specific)	
Immunomodulation		Immunomodulation

ADH, antidiuretic hormone.

Endogenous opioids are natural chemicals in the body (peptides) that interact with opiate receptors to produce natural physiological responses, including modulation of pain. β-endorphin is the endogenous opioid peptide with the highest affinity for μ receptors and is derived from proopiomelanocortin. Leucine- and methinone-enkephalin are derived from proenkephalin and are the endogenous ligands for the δ receptor. Dynorphin A is the endogenous ligand for the κ receptor and is derived from prodynorphin. The role of dynorphins remains controversial because they induce sensitization of nociceptive transmission through activation of NMDA receptors.

An endogenous opioid peptide, termed nociceptin or orphanin FQ (N/OFQ), with similar homology to dynorphin, has also been described (Meunier et al., 1995; Reinscheid et al., 1995). Orphanin precursors have been identified in the hippocampus, cortex, and numerous sensory sites (Neal et al., 1999). Exogenous N/OFQ administration results in a variety of responses ranging from analgesia to antianalgesia and opioid antagonism. However, N/OFQ antagonists have consistent analgesic properties when administered supraspinally, which may prove to be a useful target for analgesics in the future (Heinricher, 2005). However, no drugs affecting N/OFQ receptors are currently available for use, therefore these drug classes will not be addressed in this chapter.

Opioids have been characterized by the type of interaction with the opiate receptor type(s) with which they interact (μ, κ, and δ), and by the effect elicited upon binding (Figure 13.1). Opioids may be full agonists, partial agonists, antagonists, and combinations thereof. An agonist produces a dose-dependent effect which eventually plateaus with unconsciousness. A partial agonist binds to the opiate receptor, but plateaus at a sub-maximum response (less than a full agonist) despite

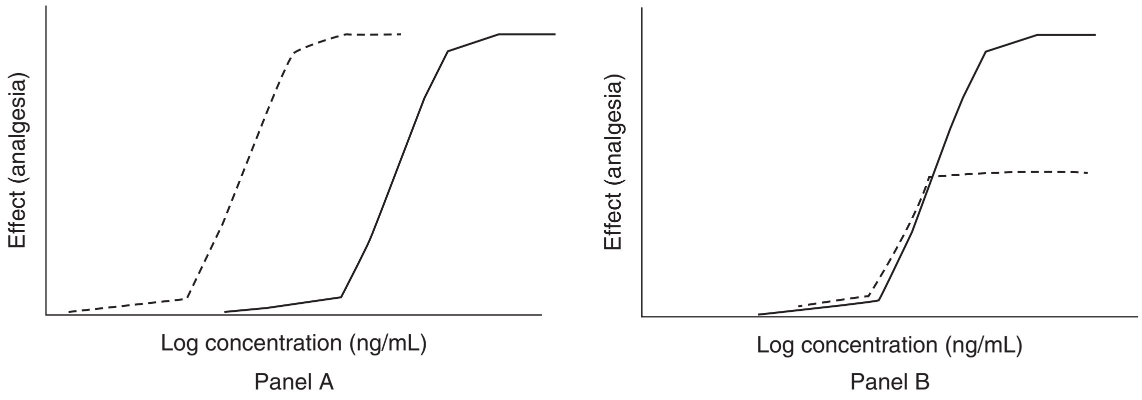


Figure 13.1 Panel A demonstrates two drugs with equal efficacy, but different potency such as fentanyl and morphine. Panel B demonstrates drugs with equal potency, but different efficacy such as fentanyl and buprenorphine.

increasing doses. The opioid antagonists are competitive antagonists which bind to the receptor but do not result in receptor activation and may displace the agonist from the opiate receptor in some cases, resulting in "reversal" of effects. Morphine is a full agonist at the μ receptor whereas buprenorphine is a partial agonist. Nalbuphine is an antagonist at the μ receptor but an agonist κ at the receptor. Naloxone is primarily an antagonist at the μ receptor but also has antagonist effects at the κ and δ receptors.

Opioid Pharmacodynamics

Analgesia

Opioids exert their primary analgesic effect by binding to spinal and supraspinal receptors (Tables 13.3 and 13.4). However, peripheral effects of opioids have been documented by local injection of opioids in joints (intraarticular injection) at doses that do not produce systemic effects and the effect is abolished when injected with naloxone (Stein et al., 1991).

When an opioid binds to spinal receptors it produces activation of G-coupled proteins (which inhibit adenylyl cyclase), activation of receptor linked K^+ ion channels, and inhibition of voltage gated Ca^{2+} channels. Presynaptic μ, κ, and δ spinal receptors are present in the dorsal horn of the spinal cord, decreasing excitatory neurotransmitter release by decreasing the rate of calcium influx. Postsynaptic μ receptors are also present in the dorsal root ganglion, which hyperpolarize the neuron by increasing potassium channel conductance resulting in a decreased propagation of the nociceptive signal.

The supraspinal opioid pathways are poorly understood, but concurrent activation with spinal receptors result in analgesic synergism (Roerig and Fujimoto, 1989; Yeung and Rudy, 1980). Synergism also exists between supraspinal μ and δ receptors when concurrently activated. Microinjection of morphine into the periqueductal gray (PAG) region results in inhibition of γ-amino butyric acid (GABA) interneurons, resulting in activation medullary pain inhibitory pathways (descending pathways) that inhibit dorsal horn nociceptors through release of serotonin (Yaksh and Tyce, 1979). Subsequent studies identified norepinephrine contributing a greater inhibitory effect than serotonin using knockout mice (Hall et al., 2011).

Full μ-opioids can provide a more profound analgesic effect than partial μ-agonists or κ-agonists for acute pain. However, partial μ-agonists and κ-agonist can still be effectively used for mild to moderate pain conditions if appropriate doses and dose intervals are chosen. The partial agonists or agonists/antagonists may be preferred in

Table 13.4 The primary sites of action for opioid and associated drugs used in veterinary medicine

	μ	κ	δ	α-2	5-HT	NMDA	M1	M3	GABA
Morphine	++	+							
Hydromorphone	++								
Oxymorphone	++								
Hydrocodone	+								
Codeine	+								
Oxycodone	++								
Heroin	++	+*							
Methadone	++			+		-			
Meperidine	++				++			–	
Fentanyl	++								
Carfentanil	++								
Etorphine	++	++	++						
Propoxyphene	+								
Buprenorphine	+								
Butorphanol	+	++							
Tramadol	+**			+	++		-**		
Nalbuphine	–	++							
Pentazocine	+	++							
Nalorphine	–	+							
Naloxone	–	-	-						-
Naltrexone	–	–	–						
Nalmefene	–	-	-						
Diprenorphine	–	–	–						

5-HT, serotonin; NMDA, *N*-methyl-D-aspartate; M1, muscarinic M1; M3, muscarinic M3.
++ agonist; + submaximal agonist; – antagonist; - submaximal antagonist; * via metabolism to morphine; ** via metabolism to *O*-desmethyltramadol.

animals that exhibit excitement or dysphoria caused by a pure μ-agonist.

Analgesia from systemic opioids are primarily limited to effects on c fiber nociceptors (slow conduction, unmyelinated nociceptors that transmit poorly localized, dull aching pain) when administered at clinical dosages. The Aδ nociceptors (fast conduction, myelinated nociceptors that transmit sharp localized pain) are minimally affected by systemic doses of opioids, which is the reason systemic opioids alone are inappropriate for invasive surgical procedures and anesthesia is required. Epidural opioids may produce high concentrations at the spinal level, which may inhibit both c-fiber and Aδ fiber nociceptors, producing greater analgesia but alone are insufficient for invasive surgical procedures. In contrast, epidural local anesthetics will completely block spinal nerve (and pain) transmission caudal to the injection site and some surgical procedures could be humanely performed.

The evaluation of analgesic response to opioids in animals is limited by other properties of the drugs (e.g., sedation) or misinterpretation of response because of dysphoria, panting, or ataxia. Innate behaviors of animals also often confound our ability to interpret responses to opioids. Clinical assessments include subjective measures that may not be indicative of pain. Placebo rates for postoperative pain studies in dogs undergoing soft tissue and orthopedic surgery have been as high as 100%. The extrapolation of experimental models of antinociceptive responses to clinical effects of these drugs is not straightforward and may overestimate or underestimate the degree and duration of analgesia. Nevertheless, many of the dosing regimens discussed in this chapter are made using information obtained from experimental models and clinical trials in the species of interest in addition to extrapolation from other species including humans.

Respiratory Depression

Opioids produce a dose-dependent respiratory depression mediated via activation of the μ receptor. Respiratory depression is produced by a decreased response to increases in carbon dioxide partial pressures ($P\text{CO}_2$). The respiratory depressant effects of opioids are well tolerated in healthy animals even when supratherapeutic doses are administered. Concurrent anesthesia or other drugs that cause respiratory depression can increase the respiratory depressant effects of opioids (Hug et al., 1981). Respiratory depressant effects may be more problematic in animals with preexisting respiratory disease (asthma, bronchitis, cor pulmonale) or increased intracranial pressure. Neonatal animals (young, healthy, and pain free) tend to be more sensitive to the respiratory depressant effects of opioids, but opioids can still be safely administered to neonates.

Opioids cross the placenta and respiratory depressant and sedative effects on the fetus can occur and should be monitored and treated appropriately. Naloxone administered sublingual (or parenterally) to newborns will reverse deleterious opioid effects.

Central Nervous System Excitation

High doses of morphine and other opioids produce excitement. Very high doses can produce convulsions. Similarly, rapid IV administration of opioids can cause CNS excitation. In dogs, morphine, 180–200 mg/kg IV, produced grand mal seizures, and convulsive changes in electroencephalogram (EEG) tracings and excitement were seen in dogs administered morphine 20 mg/kg SC (Wikler and Altschul, 1950; de Castro et al., 1979). (Note that therapeutic doses rarely exceed 0.5–1 mg/kg.) Cats respond similarly to high doses of morphine where 20 mg/kg SC produced EEG tracings consistent with convulsive activity and generalized excitement (Tuttle and Elliot, 1969).

In veterinary medicine, there are great species differences with respect to excitement caused by opioids. Dogs typically become sedate, whereas cats, horses, and other large animal species are prone to excitement. The explanation for increased susceptibility to these effects in some animals is probably related to the distribution of opiate receptors in certain regions of the brain independent of the drug's pharmacokinetics. The distribution of opiate receptors in brains of animals that are sedated from opioids (e.g., dogs) is greater than the animals that are more prone to excitement (horses). Alternatively, the excitement may be due to a release of excitatory neurotransmitters or decreased release of inhibitory neurotransmitters. Some anesthesiologists suggest that the reaction may be dopaminergic, adrenergic, or caused by decreased activity of the inhibitory neurotransmitters GABA or glycine (Werz and Macdonald, 1982). Release of acetylcholine also has been suggested as a cause of excitation (Mullin et al., 1973). In another study, it was suggested that excitement in animals may be caused by histamine release, but the doses of morphine exceeded clinically recommended doses in those studies. Morphine administration to dogs induced greater histamine release, and also more excitement compared to oxymorphone after administration of an equianalgesic dose, but the doses of morphine (2 mg/kg IV) were four times higher than recommended clinically (Robinson et al., 1988). When morphine was compared to hydromorphone in dogs (Guedes et al., 2007a), morphine caused a transient increase in plasma histamine and 1/5 dogs also exhibited neuroexcitatory behavior at 0.5 mg/kg IV. By contrast, hydromorphone did not cause increases in histamine but two dogs became excited after 0.1 mg/kg IV. These studies indicate

Table 13.5 Pharmacokinetic parameter of opioids administered IV and PO bioavailability in dogs and cats

	Half-life (h)		Clearance (ml/min/kg)		Volume of distribution (l/kg)		Oral bioavailability		IV LD_{50} (mg/kg)
	Dogs	Cats	Dogs	Cats	Dogs	Cats	Dogs	Cats	Dogs
Morphine	1.2	1.1–1.3	60	24, 42	4	2.6, 2.8	5–20 %		175
Oxymorphone	0.8	1.8	52	36.2	3.7	5.5			
Hydromorphone	0.6–1.0	0.8–1.6	68–106	28–68	4.5–5.3	3.1–4.9			
Fentanyl	3–6	~2.5	35	20	10	2.6			14
Meperidine	0.8	1.8	43	40	1.9	4.0	11 %		68
Methadone	2–4	4	30	7.2	3.5	2.3	low		29
Alfentanil	0.33	0.4	29.8	11.6	0.6	0.9			>20
Remifentanil	0.09		48–63		0.2				
Sufentanil		0.9		17.6		0.8			14
Oxycodone	1.9*						low*		
Hydrocodone	1.7		41		5.0		40–84 %		
Codeine	1.2–1.5		30–36		3.2		4–6.5 %		98
Buprenorphine	5	6.9	16	16	17.5	7.1	3–6 %		79
Butorphanol	1.6**	6.6**	57**	12.7**	8.0**	7.7**			10
Nalbuphine	1.2		46		4.6		5.6 %		140
Tramadol	0.8	2.2	55	21	3.0	3.0	4–65 %	93%	40–100

*Oral dose data, no IV dose administered; ** only IM data available, clearance is per bioavailability, volume of distribution is per bioavailability; <LOQ, no measurable plasma concentrations; LD_{50}, lethal dose 50%.

histamine alone is not a cause of opioid-induced CNS excitation.

Cats are one of the species that is more sensitive to morphine-induced CNS excitation and dysphoria. There are isolated studies in which cats have tolerated morphine doses up to 2 mg/kg (Barr et al., 2000), but lower clinical doses are generally administered to cats compared to dogs. The differences between dogs and cats are not attributed to pharmacokinetics. Although cats have somewhat lower clearance for some opiates (Table 13.5), this difference is not enough to account for opioid-induced excitement in cats.

Horses are also more sensitive to the excitement caused by this group of drugs. Because of this, pure opioid agonists such as fentanyl and morphine are not recommended to be used alone. Dose-dependent excitation and increased locomotor activity has been observed in horses and may occur before maximum analgesia is obtained with the degree of excitation dependent on the specific opioid, individual variability, and degree (or lack thereof) pain in the horses (Muir et al., 1978; Tobin et al., 1979; Mama et al., 1992). For example buprenorphine, normally well tolerated in dogs and cats, produced restlessness, excitement, head shaking, pawing, and leg shifting at a dose of 5 and 10 μg/kg IV in healthy pain-free animals that was dose dependent (Carregaro et al., 2006, 2007; Messenger et al., 2011; Davis et al., 2012). However, administration of 10 μg/kg IV in separate studies did not produce excitement, but increased locomotor activity was still observed in healthy pain-free horses (Love et al., 2015). Buprenorphine, 10 μg/kg administered as part of a preanesthetic protocol for castration, was well tolerated with no excitement, but increased locomotor activity occurred (Love et al., 2013). Morphine was investigated for its use as an anesthetic adjunct for healthy pain-free horses at doses of 0.25 and 2 mg/kg IV with isoflurane anesthesia (Steffey et al., 2003). The authors concluded that undesirable and dangerous behavior during recovery from 2 mg/kg IV morphine still persisted after 4 hours, and did not support the use of high doses of morphine as an anesthetic adjunct in this regimen. However, the recovery and effects of 0.25 mg/kg morphine were similar to the saline-treated group. When opioids have been used in painful horses, fewer adverse reactions are observed (Mircica et al., 2003; Devine et al., 2013). Only 1 of 75 clinically treated horses administered morphine (0.1 mg/kg IM) had excitation reported and it was unclear if it was drug related or simply caused by the behavior of an untrained animal (Devine et al., 2013).

Cardiovascular Effects

Most opioids have minimal effects on cardiac output in dogs, with clinically relevant doses resulting in changes ranging from slight increases to slight decreases, but methadone may produce more consistent decreases in cardiac output (Buckhold et al., 1977; Priano and Vatner, 1981; Copland et al., 1987; Maiante et al., 2009). Opioids produce bradycardia in dogs, but they usually compensate well to maintain cardiac output and treatment for the bradycardia is not routinely needed (Copland et al., 1992). Only if necessary should bradycardia can be reversed with an anticholinergic (e.g., atropine). Cardiac output is also maintained with opioids in horses and may be increased if excitatory effects occur (Muir et al., 1978; Robertson et al., 1981; Carregaro et al., 2006).

Opioids produce minimal vascular effects when administered to animals in clinically relevant doses (Barnhart et al., 2000; Pant et al., 1983; Hug et al., 1981; Kayaalp and Kaymakcalan, 1966; Priano and Vatner, 1981; Carregaro et al., 2006). Changes in mean arterial pressure range from minimal increases to minimal decreases (~10% from baseline values). Increases in plasma histamine concentrations following morphine administration are often cited as producing hypotensive effects in dogs, but histamine release is short lived (Guedes et al., 2007a) and minimal effects on blood pressure are noted following IV morphine administration. The cardiovascular effects of opioids appear to be more pronounced in anesthetized animals due to interactions with the anesthetic and when supratherapeutic opioid are administered.

The effects of opioids on myocardial blood flow are variable with some studies demonstrating increases in blood flow where others show decreases, but these studies assessed supratherapeutic doses, ≥2 mg/kg (Pant et al., 1983; Vatner et al., 1975). This is in contrast to the consistent effects in humans, coronary vasodilation and increased myocardial perfusion.

Morphine's effect on vascular tone has been used in the past as a treatment for acute cardiogenic pulmonary edema (Vismara et al., 1976). The benefits are attributed to splanchnic blood pooling, afterload reduction, or reduced breathing effort. Some investigators attributed the benefits to vasodilation caused by histamine release. This is considered a rather outdated use of morphine. Today we have potent diuretics and vasodilators to treat this condition.

Antitussive Effect

Opioids produce an antitussive effect through a central inhibition of the cough center independent of their respiratory depressant effects (Chou and Wang, 1975). Both μ- and κ- receptor agonists have been documented to produce antitussive effects (Takahama and Shirasaki, 2007). The antitussive effect is more resistant to naloxone reversal than the analgesic effect of opioids (Chau et al., 1983). Morphine, codeine, butorphanol, and methadone have demonstrated antitussive effects in dogs or cats, and tramadol additionally in cats (Rosiere et al., 1956; Cavanagh et al., 1976; Nosal'ova et al., 1991). Hydrocodone has antitussive properties in rats and people, and is commonly used in dogs for this purpose although studies definitively demonstrating its efficacy in dogs and cats are lacking (Kasé et al., 1959; Hennies et al., 1988; Homsi et al., 2002).

Dextromethorphan, which does not affect opiate receptors, is used as an over-the-counter medication to treat coughing in people. It is believed to bind to high- and low-affinity sites in the brain that are distinct from opiate receptors (Grattan et al., 1995). A steric hindrance may prevent binding to the typical opiate receptors responsible for analgesic and other effects. In people, it is more effective in spontaneous naturally occurring cough than in experimental models (Capon et al., 1996). Dextromethorphan has been shown to be an effective antitussive in dogs after IV injection (ED_{50} = 10.2 mg/kg IV), but the oral bioavailability is questionable (discussed more specifically later in Section Clinical Pharmacology, Antitussive) (Kasé et al., 1959; KuKanich and Papich, 2004a). Because it is believed that the parent drug, dextromethorphan, rather than a metabolite, is responsible for the antitussive effect (Capon et al., 1996), it is unlikely that oral administration will be effective as an antitussive in dogs.

Gastrointestinal Effects

Emetic Properties

Emesis and nausea can occur following administration of opioids via stimulation of the chemoreceptor trigger zone (CRTZ) (Mitchelson, 1992). Opioids also may stimulate central dopamine (D_2) receptors in the vomiting center to produce vomiting. Opioids appear to have differing emetic effects, with morphine causing emesis more frequently than hydromorphone or oxymorphone in dogs (Valverde et al., 2004). The emetic response is complicated by the observation that opiates also act as antiemetics in the vomiting center (Scherkl et al., 1990), with an antiemetic effect predominant for butorphanol, methadone, and fentanyl (Schurig et al., 1982; Blancquaert et al., 1986). It is not unusual to observe emesis after an initial dose of morphine or hydromorphone to a patient, but not with subsequent doses. Apomorphine administered initially will produce reliable emesis in dogs, subsequent doses do not (Khan et al., 2012). Apomorphine is discussed in more detail in Section Opioid Agonists, Apomorphine. It is commonly observed that vomiting occurs after morphine administration to pain-free animals (e.g., premedication, research animals), but vomiting is not commonly observed in animals with moderate to severe pain (e.g., trauma). These observations may be related to the antiemetic effects produced after an initial dose, the antiemetic effects produced by endogenous opioids in painful patients, or mitigation of emesis by pain.

Since the CRTZ is not protected by the blood–brain barrier, low plasma concentrations can produce emesis following oral morphine administration without producing an analgesic effect (Blancquaert et al., 1986; KuKanich et al., 2005a). In cats, morphine appears to produce more of an emetic effect as compared to fentanyl, meperidine, buprenorphine, and butorphanol (Taylor et al., 2001;

Robertson et al., 2003; Robertson et al., 2005). Hydromorphone produces signs of nausea (salivation and lip licking) in cats and may elicit emesis (Wegner et al., 2004).

Release of histamine or dopaminergic effects also may produce emesis. Apomorphine, one of the most reliable emetics in dogs, is a central-acting dopamine (DA_1, DA_2) agonist. Dopamine is one of the neurotransmitters in the vomiting center as well as in the CRTZ. Likewise, histamine is released for a short time after morphine administration to dogs (Guedes et al., 2007a) and histamine is also known as one of the neurotransmitters involved in emesis.

Gastrointestinal Motility

Opioids produce a decrease in gastrointestinal motility via both central and peripheral mechanisms. Opioid-mediated effects in the gastrointestinal tract are through the μ, κ, and δ opiate receptors. The review by DeHaven-Hudkins et al. (2008) provided details on the distribution of the receptors in the gastrointestinal tract and effects associated with stimulation, either from endogenous opioids or exogenous opioids. Opioid receptor-mediated stimulation of the gastrointestinal tract slows gastric emptying, decreases fluid secretion and increases intestinal fluid absorption, reduces propulsive motility, and increases pyloric and other sphincter tone. Expression of μ opiate receptors have been found in the submucosal plexus, myenteric plexus, and longitudinal muscle of the ileum. In healthy animals, the regulation of these receptors and their function helps to maintain gut homeostasis within the enteric nervous system by coordinating intestinal motility and intestinal secretions. Motility-induced changes by the μ, κ, and δ opiate receptors are accomplished by inhibiting Ca^{2+} channels decreasing the release of intestinal acetylcholine (Ach) and/or substance P (Cherubini et al., 1985; Galligan and Akbarali, 2014). The μ and δ opiate receptors also result in membrane hyperpolarization and decreased cAMP and protein kinase A (PKA) activity, resulting in reduced neuronal excitability. The μ and δ opiate receptors decrease intestinal fluid secretion by inhibition of chloride secretion and passive water movement into the colon. The clinical consequence is constipation or antidiarrheal effects.

After the initial emetic effect, opioids decrease gastric motility, resulting in prolonged gastric emptying. Opioids can also increase the tone of the antrum and the duodenum resulting in difficult intubation with an endoscope. The small intestinal effects of opioids initially appeared to be limited to the proximal portions; however, additional studies demonstrated effects on the ileum as well. Decreased propulsive motility and decreased intestinal, pancreatic, and biliary secretions occur in the small intestine. There is increased tone of all gastrointestinal sphincters, including those of the pancreatic duct. However, increases in rhythmic, segmental, nonpropulsive contractions also occur, which enhances reabsorption of water.

Morphine initially stimulates large bowel motility and defecation often occurs shortly after administration in dogs and cats (Tuttle and Elliot, 1969; Barnhart et al., 2000; Lucas et al., 2001). Following initial defecation, opioids produce a decrease in colonic propulsive motility and secretions, but increases in nonpropulsive rhythmic contractions occur similar to that in the small intestine. Therefore the passage of colonic contents is delayed and the fluid content is decreased. The decreased gastrointestinal motility can result in constipation, an adverse effect, or can be used therapeutically as a treatment for diarrhea.

The decrease in intestinal motility is profoundly important for horses. Horses treated for pain are often prone to ileus. Administration of opioids to horses will decrease production of feces, decrease intestinal sounds, and potentially lead to ileus and constipation (Boscan et al., 2006). The effects are best known for morphine, but also can occur with butorphanol, an agonist/antagonist (Sellon et al., 2001). The butorphanol effects are dependent on method of administration because when butorphanol was administered by a constant rate infusion, the intestinal effects were less compared to an IV bolus injection (Sellon et al., 2001).

Urinary Tract

Opioids produce a variety of effects on the urinary tract. μ-agonists increase the tone of urinary sphincters. Micturition is inhibited, which may result in urine retention and may be mediated by spinal as opposed to local mechanisms (Drenger et al., 1986, 1989).

Mu-receptor agonists decrease urine production. As discussed by Robertson et al. (2001), although it has been documented that morphine produces an antidiuretic effect and decrease in urine output, the exact mechanism is unclear. It is not known whether it is caused by a release of arginine vasopressin (AVP) (also known as antidiuretic hormone), or via some other mechanism. In contrast, κ opioid agonists produce a diuretic effect as a result of decreased AVP concentrations (Craft et al., 2000). For a drug such as morphine that acts as an agonist for both receptors, the net effect observed clinically is diuresis in most situations. This is complicated by the effect on the urinary bladder. Morphine increases the tone of the sphincter of the urinary bladder, making it more difficult for some animals to voluntarily urinate. Morphine also inhibits the urinary voiding reflex. As a result of these effects hospitalized animals should be monitored and interventions used to facilitate bladder emptying when necessary.

Immune System Effects

The effects of opioids on the immune system are complex. The immune system effects are likely related to the specific opioid administered, dose, and the duration of treatment. A complex interaction of the immune system, sympathetic nervous system, endocrine system (hypothalamic–pituitary–adrenal axis), and direct effects on leukocytes occur. Immunosuppressive and immuostimulant properties have been identified depending on the experimental conditions. Some painful patients may benefit from opiate effects on immune function (Page and Ben-Eliyahu, 1997). Extrapolations of the effects of opioids on the immune system from studies on human opioid abusers are likely inaccurate for veterinary patients because studies in humans are in the context of high dose administration, chronic use, and tolerance of human opioid abusers.

Tolerance and Dependence

Although these traits are often associated with opioid use in humans, they have been well documented in animals. Tolerance and dependence can be demonstrated in animals, but opioids are seldom used for a long-enough period of time at large-enough dosages in veterinary patients for this issue to have clinical consequences. The NMDA receptor may play a role in opioid tolerance and dependence as treatment with NMDA antagonists attenuate withdrawal symptoms in experimental settings (Yeh et al., 2002). The consequence of tolerance is that drug dosages may need to be increased with chronic administration of opioids.

Opioids administered for a period as short as 5–7 days may result in dependence in dogs. In one study in dogs, constant rate infusion to dogs at a rate of 1–5 mg/kg/day produced physical dependence by day 8 (Yoshimura et al., 1993). Withdrawal signs can be elicited in dogs after dependence with injections of naloxone. Signs of withdrawal in dogs include nausea, aggression, vocalization, vomiting, hyperactivity, hyperthermia, tremors, and salivation. Withdrawal can also be elicited from administration of a μ antagonist or partial agonist such as butorphanol or buprenorphine in opioid-dependent dogs (Yoshimura et al., 1993).

Therefore, if an animal has been receiving continuous opioids drugs for longer than 5–7 days, withdrawal signs may occur after administration of an antagonist or partial agonist. However, clinical experience suggests that discontinuing the opioid rarely causes withdrawal signs for durations of administration less than a week. In contrast, withdrawal signs have occurred clinically when butorphanol (μ-antagonist) was administered to a dog after 1 week of morphine administration. Decreasing the dose of the μ agonist to wean a patient off an opioid or decrease opioid effects is a better strategy than switching to a partial agonist or antagonist.

Table 13.6 Chemical properties of opioids

Opiate	LogP[a]	Solubility (mg/l)	pKa
Meperidine	2.72	6550	8.59
Buprenorphine	4.98	0.0168	8.31
Butorphanol	3.3	0.16	10.7
Sufentanyl	3.95	76	8.86
Fentanyl	4.05	200	8.77
Methadone	3.93	48.5	8.94
Hydromorphone	0.9	4.39	8.59
Oxymorphone	0.83	2.4×10^4	8.17
Morphine	0.89	149	8.21

[a] LogP, log of the octanol/water partition coefficient.

Opioid Pharmacokinetics

Opioids tend to be well absorbed when administered orally, intramuscularly, or subcutaneously. However, because of substantial first-pass metabolism the oral administration of most opioids results in poor bioavailability and erratic plasma concentrations in animals. Administration of opioids per rectum to animals result in a minimal increase in bioavailability over oral administration, since the majority of drug absorbed by this route of administration is still subject to first-pass hepatic metabolism due to uptake into the portal vein. Nasal and oral transmucosal administration of opioids can bypass first-pass hepatic metabolism. However irritation, the volume of drug to be delivered, dosing frequency, and species specific differences have limited the clinical effectiveness of transmucosal administration to buprenorphine in cats.

Opioids are well distributed throughout the body. Opioids are bound to plasma proteins in variable amounts from low to high protein binding depending on the individual drug. The opioids are lipophilic and weak bases. A comparison of the lipophilicity, designated by the octanol/water partition coefficient, is shown in Table 13.6. The higher the partition coefficient, the greater is the lipophilicity. All are weak bases (some have acidic groups also) with pKa values above 8. This indicates that lipophilicity increases at pH values of 8 and higher. These physiochemical properties favor intracellular accumulation. Consequently, opioids have a large volume of distribution, which greatly exceeds total body water. Additionally, administration of opioids with high lipophilicity, such as fentanyl (the highest Log P in Table 13.6), can produce rapid redistribution from tissues that are highly perfused, such as the central nervous system, to tissues that are less perfused, such as muscle and fat. The result is a more rapid decrease in concentrations (and effect) in the target organs in comparison to total body elimination. Repeated doses or a constant rate infusion may result in drug accumulation with an increased effect and slower than expected recovery. This phenomenon is similar to that seen with repeated doses of thiobarbiturates.

Systemic clearance of most opioids in animals is high. As seen in Table 13.5, clearance approaches or exceeds hepatic blood flow for most drugs, designating these as *high clearance* drugs. Most opioids are metabolized to more polar compounds and subsequently excreted in the urine or bile, but a small amount of unchanged drug may be eliminated in the urine and feces. Conjugation reactions are the primary means for some opioid metabolism (e.g., morphine and codeine), although cytochrome P450 (CYP) mediated metabolism can also occur (e.g., fentanyl, methadone), which are compound and species specific. Some of the conjugated products (e.g., glucuronide conjugates of opiates) are pharmacologically active, but because of their higher polarity may not cross the blood–brain barrier. Extrahepatic metabolism of morphine occurs in dogs, resulting in clearance rates exceeding the combined hepatic blood flow and glomerular filtration rates. Glucuronide conjugation is the primary conjugation pathway of morphine in most animal species except cats, which are a species deficient in some glucuronidation mechanisms (Court and Greenblatt, 1997). Sulfate conjugation is the primary means of morphine metabolism in cats. Despite differences in metabolic pathways, cats effectively metabolize morphine with total body clearance approximating the expected hepatic blood flow rate indicative of a high extraction drug (Table 13.5). Oxidative metabolism, mediated by CYP, appears to be the primary mechanism of fentanyl (and derivatives), methadone, and meperidine metabolism. Remifentanil is metabolized by tissue and plasma esterases and produces an extremely short half-life. The pharmacokinetic parameters of opioids in dogs and cats are presented in Table 13.5.

Clinical Pharmacology

Analgesia

Opioids are commonly used for analgesia and to relieve pain and anxiety from painful syndromes. Clinically, it is observed that there is great variability in response among patients, and between drugs. High variability has been recorded for pharmacokinetics in a species and analgesic responses varied by many fold in experimental studies. Therefore, careful observation and a willingness to adjust doses (or switch drugs) in individual patients is important to successful pain management.

Severe pain, often associated with trauma, should be treated with μ opioids agonists with high intrinsic activity (e.g., morphine, hydromorphone, fentanyl, methadone). Although some references have stated opioids should not be administered to painful animals in shock, there is no basis for this recommendation. Pain can contribute markedly to the adverse and life-threatening effects associated with shock and opioids often improve the hemodynamics and tissue perfusion of painful patients. As previously stated, the hypotensive effects of intravenous opioids (including morphine) are minimal when administered in clinically relevant doses, and concurrent supportive measures, such as fluid therapy, are essential to successful therapy in these patients. Mild to moderate pain (e.g., soft tissue surgery) has been managed with μ opioid agonists, partial agonists, mixed agonist–antagonists, or tramadol. The efficacy (and lack thereof) and variability of oral tramadol are discussed later.

Preemptive analgesia, dose administration prior surgical or a painful stimulus and continued into the postoperative period, provides more effective analgesia than holding off administration of opioids to the postoperative period in dogs (Lascelles et al., 1997). This is thought to occur by decreasing central sensitization (also known as the wind-up phenomenon).

Chronic and neuropathic pain appear to be less responsive to opioid analgesia. Higher doses of opioids in humans are needed for controlling neuropathic pain (Rowbotham et al., 2003). For neuropathic pain, it may be more effective to use a multimodal approach utilizing opioids in combination with other drug classes such as NMDA antagonists (e.g., ketamine), gabapentin, local anesthetics (e.g., lidocaine), or α-2 agonists (e.g., dexmedetomidine) appear to be more effective in refractory cases.

Antitussive

Butorphanol tablets (Torbutrol) are approved by the FDA for antitussive use in dogs. Hydrocodone is also routinely prescribed for its antitussive effects in dogs (see Chapter 48). Codeine exhibits poor bioavailability in dogs and may not be effective when administered orally. (Codeine has questionable antitussive properties in people as well (Takahama and Shirasaki, 2007).) Tramadol tablets have demonstrated effectiveness as an antitussive in experimental models in cats due to the formation of an opioid metabolite, but have yet to be evaluated clinically. All opioid agonists are expected to be effective antitussives when administered parenterally. As mentioned previously, oral dextromethorphan – a nonopiate that is a common ingredient in human over-the-counter cough medications – is probably not an effective antitussive in dogs because of the low oral bioavailability and lack of activity from metabolites.

Congestive Heart Failure

As mentioned Section Opioid Pharmacodynamics, Cardiovascular Effects, routine use of morphine is no longer recommended in treatment of congestive heart failure in animals due to variable effects on coronary blood flow. Likewise, although used in the past as a treatment for acute cardiogenic pulmonary edema (Vismara et al., 1976), this is no longer a common use.

Antidiarrheal

Opioids are effective antidiarrheal drugs as they decrease gastrointestinal secretions, decrease propulsive gastrointestinal contractions, and increase nonpropulsive segmental gastrointestinal contractions (see Chapter 46). Loperamide is the most commonly recommended opioid for the treatment of diarrhea because of reliable effects, availability over the counter (not a controlled substance), low cost, and safety profile. Loperamide is p-glycoprotein (p-gp) substrate and as a result is excluded from the CNS in normal animals. However, if concurrent p-gp inhibitors (e.g., cyclosporine, ketoconazole, spinosad) are coadministered or the animal is deficient in p-gp (some Collie dogs, Collie related breeds, and other herding breeds) than sedation, dysphoria, and other marked CNS opioid effects may be noted for prolonged periods of time, which may exceed 24 hours (Hugnet et al., 1996). Diphenoxylate in combination with atropine is also marketed as an antidiarrheal for humans, but is not used frequently in veterinary medicine as it has not demonstrated increased efficacy over loperamide. Diphenoxylate is a prescription drug, and is a schedule V controlled substance requiring special record keeping.

Sedation Anesthesia

Opioids are commonly coadministered with sedatives, tranquilizers, and anesthetics. Additive or synergistic sedative and analgesic effects occur when opioids are used in combination with sedatives and anesthetics. Combinations of these drug classes result in decreased dosages of the individual drugs with the potential for decreased adverse effects. Additionally, preemptive analgesia increases the effectiveness of postoperative analgesics, decreasing opioid consumption, adverse effects, and cost. A more thorough review of sedation and anesthesia drug combinations is presented in Chapters 12 and 14.

Contraindications, Warnings, and Drug Interactions

Head Trauma

As a drug class opioids exhibit a wide safety margin. Cautious use of opioids in patients with head trauma is warranted as respiratory depression may increase blood carbon dioxide concentrations resulting in cerebral vasodilation and worsening effects of cerebral edema. However, use of lower doses more frequently or constant rate infusions will mitigate some of the adverse effects as they are concentration (peak) proportional.

Temperature Regulation

Opioids have varying effects on temperature regulation depending on the species (Adler et al., 1988). Panting occurs after administration of some opioids in some dogs. Hydromorphone and oxymorphone caused 60% of the dogs to pant in one study (Smith et al., 2001). Panting can be aggravating, especially in animals being induced with inhalant anesthetics or undergoing diagnostic procedures such as radiography or ultrasound, but is usually self-limiting. It is not related to effects on the respiratory center, but is a reaction to the effect of opioids on the thermoregulatory center of the hypothalamus, whereby the body's set point is typically decreased by 1–3°F and dogs pant to lower their temperature. The depression of a dog's body temperature is a consistent finding in published studies (Lucas et al., 2001).

In contrast, administration of opioids has produced increase in body temperature in cats, horses, cattle, swine, and goats. Hydromorphone has been the best documented to produce this reaction, but other opioids are also associated with hyperthermia including morphine, buprenorphine, and butorphanol (Posner et al., 2007; Niedfeldt and Robertson, 2006). In published studies hyperthermia associated with hydromorphone has been as high as 41–42°C (107–108°F) in cats, for as long as 5 hours. The authors of these studies warn of high temperatures affecting postoperative recovery in cats when opioids are used for analgesia or perioperative sedation.

Interactions Between Opioid Drugs

There has been a controversy as to whether or not administration of opioid pure agonists with opioid agonist/antagonists will produce an interaction that diminishes the analgesic effect of the combination. There is a possibility that drugs such as butorphanol and pentazocine, which have antagonistic properties on the μ receptor, will partially reverse some effects of pure agonists such as morphine if they are administered together. The clinical relevance of this antagonism has been debated. There are few reports that have documented that such a combination is antagonistic. Reversal of the pure μ opioids can occur and acute pain or signs of opioid withdrawal may be precipitated following concurrent administration (Lascelles and Robertson, 2004).

One use of antagonistic qualities of the opioids has been to administer a μ-receptor antagonist, or partial agonist (e.g., buprenorphine) to lessen some of the dysphoric effects caused occasionally from pure μ-receptor agonists such as morphine or fentanyl, while still providing some degree of analgesia. In dogs, butorphanol can partially reverse the effects of oxymorphone (Lemke et al., 1996). Butorphanol may reverse some respiratory depression and sedation from pure agonists, but some of the analgesic efficacy is preserved. In dogs that received butorphanol for postoperative pain associated with orthopedic surgery, there was no diminished efficacy from subsequent administration of oxymorphone (Pibarot et al., 1997). However, in another study, some dogs that had not responded to butorphanol after shoulder arthrotomy responded to subsequent administration of oxymorphone, but as expected the oxymorphone dose required to produce an adequate effect was higher than what would be required if oxymorphone was used alone (Mathews et al., 1996).

Other results have been observed in cats. Increased analgesia, decreased analgesia, or no effect has been observed from combinations of agonists and antagonists. When butorphanol and oxymorphone were administered to cats in combination, there was greater efficacy than either drug used alone, with no observed antagonistic effects (Sawyer et al., 1994; Briggs et al., 1998). On the other hand, the administration of hydromorphone with butorphanol may decrease the analgesic from either drug used alone (Lascelles and Robertson, 2004). When butorphanol, buprenorphine, and the combination of both drugs were administered to cats all three treatments provided similar antinociceptive effects (Johnson et al., 2007). Although butorphanol is a κ-receptor agonist/μ-receptor antagonist, and buprenorphine is a κ-receptor antagonist/μ-receptor partial agonist, there was no antagonism detected. However, the design of the study may have prevented a more sensitive delineation of treatment effects. In general, administration of combinations of agonists and antagonist is not recommended due to variable and potential antagonistic effect.

There are data available in the human literature documenting enhanced analgesic effects and reduced tolerance by coadministering ultra-low-dose opioid antagonists with μ-agonists. The doses of the antagonists are lower than needed to completely antagonize the agonist. For example, naloxone administered as a constant rate infusion (0.25 μg/kg/h) significantly reduced the morphine consumption and gastrointestinal adverse effects in hysterectomy patients (Movafegh et al., 2012). Even lower doses have been examined in experimental animals and humans (la Vincente et al., 2008). In these studies an "ultralow naloxone" dose is in the range of 3.5 μg total dose for a 70 kg human (0.05 μg/kg). When combined with an opioid, these low doses produced significantly greater enhanced analgesia with fewer adverse effects than when the opioid was administered alone. This is in contrast to the typical naloxone dose of 0.4 mg total dose to antagonize opioid agonists in humans. Although this is an area worthy of further study in veterinary medicine, optimized doses, drug combinations, and demonstrated benefits in veterinary species are lacking.

Interactions with Drug Clearance

Opioids are high clearance drugs in animals; therefore, they rely more on hepatic blood flow than activity of liver enzymes for clearance. Diseases or conditions that impair hepatic blood flow (e.g., cardiac disease, hypotension, hypovolemia) may substantially affect opioid drug clearance. Interactions with hepatic microsomal cytochrome P450 enzymes (CYP), protein binding, or loss of hepatic function are not expected to affect most opioids' drug clearance, but some exceptions exist. The pharmacokinetics of methadone are significantly affected when combined with chloramphenicol (KuKanich and KuKanich, 2015), resulting in prolonged methadone drug exposure, higher maximum concentrations and pronounced opioid effects. The effects of chloramphenicol on other opioids likely metabolized by CYP (e.g., fentanyl, meperidine) have not been reported, but interactions could occur. An opioid not metabolized by CYP (e.g., morphine) would be a better choice until more data are available.

Renal impairment decreases the clearance of morphine glucuronide metabolites with a resultant metabolite accumulation. However, the importance of opioid glucuronide metabolites has not been demonstrated in animals and clinical consequence of metabolite accumulation has not been demonstrated in animals.

Interactions with Other Analgesics and Antidepressants

The most common drug–drug interactions with opioids occur when combined with tranquilizers/sedatives and is often used clinically to increase sedation. There is a specific interaction described in people between monoamine oxidase (MAO) inhibitors and meperidine. The interaction has been most commonly observed with older nonselective and irreversible inhibitors of MAO. The use of these drugs together has caused an unpredictable, and a sometimes fatal reaction. The reaction includes excitation, sweating, rigidity, coma, and seizures. This reaction is due to the effect of meperidine (and its metabolite normeperidine) on serotonin turnover in conjunction with the MAO inhibitors decreasing metabolism of serotonin, resulting in serotonin syndrome. This is discussed in more detail later in Section Opioid Agonists, Meperidine(Pethidine). Other analgesics that may be affected include methadone and tramadol as they also both affect serotonin turnover. The interaction with opioids that do not affect serotonin turnover (e.g., morphine, hydromorphone, fentanyl, butorphanol, etc.) is unlikely. It has been suggested if an animal is receiving an MAO inhibitor, that a test dose of the opioid should be administered. If there is no adverse reaction, subsequent doses can probably be administered safely. Although nonselective MAO inhibitors (e.g., type A and B MAO inhibitors) are rarely used in veterinary medicine for treatment of depression as they are in people, other drugs with MAO-inhibiting qualities are used in animals. For example, selegiline, a specific MAO-type B inhibitor (deprenyl, Anipryl), is used in dogs to treat canine hyperadrenocorticism and cognitive disorder (discussed in Chapter 18). A study assessing the effects of selegiline on oxymorphone and butorphanol in dogs failed to demonstrate a detrimental interaction (Dodam et al., 2004). Amitraz (Mitaban) is also a MAO-inhibitor and is found in pet collars and dips to prevent and treat mite infections in animals (see Chapter 43). Although no reactions in animals have been described between amitraz or selegiline and opioid analgesic drugs, one should be aware of a potential interaction and the animal monitored closely for adverse effects.

Uses In Food-Producing Animals

Use of other opioids in food producing animals is permitted by the Animal Medicinal Drug Use Clarification Act (AMDUCA) if an adequate withdrawal interval can be determined. Therefore the Food Animal Residue Avoidance Databank (FARAD) or a reliable reference (Papich, 1996, 2016) should be consulted prior to opioid administration to food producing species in order to obtain the most current withdrawal interval recommendations.

Opioid Agonists

Morphine

Morphine is commonly used in veterinary medicine due to its safety, efficacy, tolerability, and low cost, despite the development of newer opioids. Current dosage recommendations are presented in Table 13.7. Morphine is effective for mild to severely painful conditions. Intravenous administration results in dose-dependent increases in plasma histamine (Thompson and Walton, 1964), but at doses <0.6 mg/kg IV, no significant changes occurred in blood pressure in awake dogs (Guedes et al., 2006, 2007a). Higher doses, 3 mg/kg IV, resulted in higher plasma histamine and significant decreases in blood pressure in pentobarbital anesthetized dogs (Muldoon et al., 1984). Histamine release may possibly account for transient excitement, but it is short lived (Robinson et al., 1988; Guedes et al., 2007a). Morphine exhibits moderate protein binding in most species approximating 30%. Morphine is also produced endogenously in both humans and animals in response to inflammation and stress (Brix-Christensen et al., 2000; Yoshida et al., 2000).

Dogs

Even though the clinical morphine dose is much smaller (Table 13.7), at a dose of 3 mg/kg it showed a wide safety profile in dogs. At high doses of 40 mg/kg it produced severe coronary perfusion effects, 180 mg/kg eliciting seizures in ventilated dogs, and death at doses higher than 200 mg/kg in dogs (de Castro et al., 1979; Muldoon et al., 1984). The reported IV LD_{50} of morphine (HCl) in dogs is 175 mg/kg (Kasé et al., 1959). The pharmacokinetics of morphine were reported in several studies and are summarized in Table 13.5. Constant rate IV infusion of morphine in dog studies demonstrate the high clearance rates and need for high infusion rates (KuKanich et al., 2005c; Lucas et al., 2001; Guedes et al., 2007b). Because of its low oral and rectal bioavailability, morphine should be administered parenterally in order to consistently achieve therapeutic concentrations. The low lipophilicity in comparison to other opioids (Table 13.6) may account for the low rectal and transmucosal absorption. Morphine is also minimally absorbed following transdermal administration of a pluronic lecithin organogel formulation (Krotscheck et al., 2004). Morphine is primarily metabolized by hepatic and extrahepatic glucuronide

Table 13.7 Morphine dosages (only parenteral doses are listed, oral administration is not recommended)

	IV	IV infusion	IM/SC
Dog	0.25–0.5 mg/kg q 2–3 h	0.1–0.2 mg/kg/h	0.25–1 mg/kg q 2–4 h
Cat	0.1–0.25 mg/kg q 2–3 h	0.05–0.1 mg/kg/h	0.1–0.5 mg/kg q 2–4 h
Horse	0.1–0.2 mg/kg q 8 h	NR	0.1–0.2 mg/kg q 8 h
Sheep			≤ 10 mg total dose
Goat			≤ 10 mg total dose
Swine			0.2–0.9 mg/kg
Rat / mouse			2–5 mg/kg q 4 h
Guinea Pig / Hamster			2–5 mg/kg q 4 h
Rabbit			2–5 mg/kg q 4 h
Primates			0.5–2 mg/kg q 4–6 h
Ferrets			0.5 mg/kg q 4–6 h

NR, not recommended.

conjugation to morphine-3-glucuronide and morphine-6-glucuronide. Morphine-3-glucuronide (M3G) has little analgesic effect and may be responsible for some of the adverse effects. Morphine-6-glucuronide (M6G) has analgesic effects that are greater than the parent drug, morphine, when administered by intracerebroventricular injection. However, little of the M6G metabolite was found in canine studies (Yoshimura et al., 1993; KuKanich et al., 2005c) and its ability to penetration the CNS may be limited by the low lipophilicity in comparison to the parent drug.

A 30 to 45-minute lag time to maximum effect after 0.5 mg/kg IV morphine occurs primarily due to slow penetration into the CNS, but clinically relevant analgesia occurs within 5–15 minutes. Duration of analgesia is from 1 to 4 hours following administration of 0.25–1 mg/kg IV, IM, SC, with the length of effect approximately proportional to the dose (KuKanich et al., 2005b, 2005c). Due to the rapid and complete absorption of morphine administered IM and SC the dosing interval is the same as for IV administration.

Adverse effects associated with morphine are typical of opioids and can include sedation, emesis, hypothermia, constipation, dysphoria, pain on injection, defecation, panting, miosis, respiratory depression, and diuresis. Morphine produced vomiting in 75.7% of dogs after a dose of 0.5 mg/kg IM (Koh et al., 2014), and in 80% of dogs after a dose of 1 mg/kg (Wilson et al., 2005). Vomiting may be more frequent than other opioids, but rapid tolerance to this adverse effect develops and emesis rarely occurs with repeated administration (Valverde et al., 2004). Intravenous morphine produced transient increases in histamine release in dogs compared to other opioids, but in clinically relevant dosages minimal clinical effects are expected (Guedes et al., 2007a). Neonates are more sensitive to the respiratory depressant effects of morphine, but dogs as young as 2 days old required doses of 1 mg/kg before significant respiratory depression occurred (Bragg et al., 1995). A study examining the effect of ketoconazole on morphine pharmacokinetics in Greyhounds indicated no dose adjustments are needed for this breed or when ketoconazole is administered concurrently with morphine (KuKanich and Borum, 2008a).

Cats

Morphine is well tolerated in cats, rarely producing CNS excitation when administered in clinically recommended doses. Laboratory studies administered cats morphine at a dose of 3 mg/kg SC which did not produce excitement, but 5–10 mg/kg SC produced signs of CNS excitation and 20 mg/kg SC produced consistent "morphine mania" after administration (Sturtevant and Drill, 1957.) Morphine, 2 mg/kg IM, was well tolerated with mydriasis, sedation, pacing, pouncing, emesis, and fascination with water being noted as adverse effects, but CNS excitation and "morphine mania" were not noted in the study (Barr et al., 2000, 2003). Clinically recommended morphine doses, 0.2 mg/kg, produced vomiting in healthy cats, but otherwise did not produce dysphoria or excitement (Steagall et al., 2006). Instead, the cats exhibited euphoria with purring, kneading, and increased affection.

The primary metabolite of morphine in cats are sulfate conjugates because cats are deficient in glucuronidation of some compounds compared to other species (Yeh et al., 1971). The lack of glucuronide conjugate formation in cats is not unexpected, but the metabolism is still rapid in cats and indicative of a high extraction drug. Pharmacokinetic studies in cats have indicated a smaller volume of distribution and slower clearance as compared to dogs, with the elimination half-life being nearly identical (KuKanich et al., 2005a; Taylor et al., 2001; Pypendop et al., 2014). Assuming cats respond to the analgesic effects of morphine at similar concentrations as other animals, 0.2–0.3 mg/kg IV, IM, SC every 3–4 hours would produce similar plasma concentrations as other animal species.

Studies examining the effects of morphine on feline immunodeficiency virus(FIV) infected cats produced unexpected results. Morphine, 1–2 mg/kg administered once daily subcutaneously on 2 consecutive days per week, significantly reduced the clinical signs associated with FIV infection including delayed lymphadenopathy, CNS deterioration, and a trend towards decreased viral load (Barr et al., 2000, 2003). Clinical trials in naturally infected cats have not been conducted.

Horses

Morphine has been safely administered to horses under some conditions, but reports of excitement, behavior changes, and sustained increased locomotor activity have limited its routine use for pain control. Reports of excitement following morphine administration to horses may be caused by doses that were too high or lack of concurrent sedatives (e.g., α_2-agonists or tranquilizers) administered. In comparison to other species, horses seem to be the most prone to excitation/ increase locomotor activity as an adverse effect. Morphine, 0.1–0.25 mg/kg IV, as a sole agent has been administered with no reported excitement and resulted in decreased anesthetic consumption, produced minimal hemodynamic, and minimal blood gas effects (Devine et al., 2013; Clark et al., 2005; Bennett et al., 2004; Steffey et al., 2003; Combie et al., 1983; Muir et al., 1978). However higher doses, 0.66 mg/kg and above, resulted in adverse effects of blood gas measurements, dysphoria, and excitement, and therefore should be avoided (Brunson and Majors, 1987; Kalpravidh et al., 1984). Coombie et al. (1983) recommended morphine should not be administered less than 7 days prior to a race as it is detectable in the urine at

144 hours (6 days). The regulation of drugs used in racing animals is discussed in more detail in Chapter 57. Repeated doses of morphine should be used extremely cautiously in horses due to its constipating effect and the potential for impaction and colic.

Ruminants

The clinical use of morphine in ruminants in rare. The lack of residue depletion studies, controlled substance status, and the potential to decrease rumen contractions have limited its use.

Swine

Morphine has been associated with CNS excitation in swine, but like other species is likely to be related to the dose administered. The clinical use of morphine in swine is limited by the lack of residue depletion studies and controlled substance status, but with increasing numbers of swine as companion animals (e.g., pot-bellied pigs) and in research settings the use of morphine in this species may increase.

Small Mammals

Morphine has been used in rats, mice, guinea pigs, and ferrets as an analgesic, preanesthetic, and sedative. Analgesic effects are rapid following IM and SC administration typically occurring within 15 minutes and lasting up to 4 hours, similar to other species.

Rabbits

Morphine has been previously reported to produce profound sedation in rabbits when administered 8 mg/kg IM as a preanesthetic. However, pharmacokinetic studies indicate analgesic dosages are probably in the range of 2–4 mg/kg IM or SC every 4–6 hours.

Fish

Morphine has been used in fish to control pain (Sneddon, 2003). Fish not only feel pain (Sneddon et al., 2003), but they also appear to have opiate receptors. However, one study in which morphine was administered at high doses also reported that there were significant adverse cardiovascular effects (Newby et al., 2007). Fish appear to have slower clearance rates than mammals. In one study (Newby et al., 2006) clearance rates were 25 to 50 times slower than dogs, and mean residence times were 7 hours and 27 hours in two species of fish.

Nonhuman Primates

Nonhuman primates require relatively large doses of morphine, 1–2 mg/kg, for sedation. However nonhuman primates also appear to be relatively resistant to respiratory depression at these dosages despite high plasma concentrations (Lynn et al., 1991). Analgesic dosages are more likely in the range of those recommended for dogs.

Hydromorphone

Hydromorphone is a morphine derivative with similar lipophilicity, but greater potency compared to morphine (Murray and Hagen, 2005) (Tables 13.6 and 13.8). Hydromorphone is approximately seven times more potent than morphine with a similar duration of effect. Hydromorphone has high intrinsic activity at the μ-opioid receptor. The effectiveness for mild to severe pain is similar to morphine and oxymorphone with equipotent doses (Smith et al., 2001) (0.11 mg/kg for oxymorphone; 0.22 mg/kg for hydromorphone). Hydromorphone produced less of an emetic effect than morphine in some studies, but a high incidence in other studies. As cited above for morphine, vomiting rates are high. After hydromorphone administration the incidence of vomiting was 25% in one study (Hay Kraus, 2014a), but much higher in other studies (Claude et al., 2014; Hay Kraus, 2014b). Hydromorphone produced less histamine compared to equipotent morphine doses (Guedes et al., 2006). In dogs, hydromorphone pharmacokinetics were reported between 0.1 and 0.5 mg/kg IV, SC (Guedes et al., 2008; KuKanich et al., 2008a). The pharmacokinetics were similar to morphine in dogs (Table 13.5), with a short half-life, high volume of distribution, and high clearance that exceeded hepatic blood flow. Significant differences in the pharmacokinetics of hydromorphone occurred between 0.1 and 0.5 mg/kg with the higher dose resulting in significantly decreased clearance and prolonged half-life (KuKanich et al., 2008a). Antinociception was detected for at least 2 hours at 0.1–0.2 mg/kg IV, and may be up to 4–6 hours depending on the dose, degree of pain, and individual animal variability (Guedes et al., 2008).

The pharmacokinetics and pharmacodynamics of hydromorphone have been investigated in cats (Table 13.5) in which a rapid clearance and short half-life were determined, similar to morphine (Wegner et al., 2004; Wegner and Robertson, 2007; Pypendop et al.,

Table 13.8 Comparable potency of opioids

Drug	Brand Name	Potency [a]
Morphine	Generic	1
Codeine	Generic	0.1
Meperidine	Demerol	0.16
Propoxyphene	Darvon	0.16–0.3
Butorphanol	Torbugesic	1–7
Hydrocodone	Vicodin	6
Oxycodone	Percocet, Oxy-Contin	3–6
Oxymorphone	Numorphan	10–15
Hydromorphone	Dilaudid, generic	8
Buprenorphine	Buprenex	25
Fentanyl	Sublimaze	100

[a] Potency is based on relative potency in comparison to morphine.

Table 13.9 Hydromorphone dosages (only parenteral doses are listed, oral administration is not recommended)

	IV	IV infusion	IM/SC
Dog	0.05–0.1 mg/kg q 2–4 h	0.02–0.04 mg/kg/h	0.1 mg/kg q 2–4 h
Cat	0.05–0.1 mg/kg q 2–4 h	0.02–0.03 mg/kg/h	0.05–0.1 mg/kg q 2–4 h
Horse	0.01–0.02 mg/kg once	NR	0.01–0.02 mg/kg once
Rat / mouse			0.3–0.7 mg/kg q 4h
Guinea pig / hamster			0.3–0.7 mg/kg q 4h
Rabbit			0.3–0.7 mg/kg q 4h
Primates			0.1–0.3 mg/kg q 4–6h
Ferrets			0.05–0.1 mg/kg q 4–6h

NR, not recommended.

2014). Although analgesic effects produced had a duration of up to 8 hours in the experimental model, it is unlikely these correlate with clinical analgesia. The recommended starting dosage interval for hydromorphone is the same as morphine, every 2–4 hours in dogs and cats, but may be up to 6 hours depending on pain severity and individual animal differences. Hydromorphone is associated with hyperthermia in cats, but hyperthermia has been reported with other opioids including morphine, buprenorphine, and butorphanol (Posner et al., 2007; Niedfeldt and Robertson, 2006). Hyperthermia associated with hydromorphone has been as high as 41–42°C (107–108°F) in cats, and can last for as long as 5 hours.

Hydromorphone is available as oral syrup and tablets as hydromorphone hydrochloride. Although the pharmacokinetics of the oral formulations have not been reported for dogs or cats, the oral bioavailability is expected to be low or negligent because of the high first-pass clearance. Hydromorphone hydrochloride injection is widely available and commonly used in veterinary medicine. Current dose recommendations for hydromorphone are presented in Table 13.9.

Oxymorphone

Oxymorphone is a morphine derivative with 10 times the potency of morphine (Table 13.8) and similar lipophilicity. Oxymorphone produces less emesis, nausea, and sedation than morphine and hydromorphone when administered at equipotent doses to dogs and cats. Oxymorphone also causes less histamine release in dogs (Robinson et al., 1988; Smith et al., 2001). Oxymorphone is primarily eliminated as drug conjugates (56% of total dose) with small amounts eliminated unchanged in the urine (5.3%) or as 6-keto reduction metabolites (1.6%) in dogs. The pharmacokinetics of oxymorphone in dogs are similar to other opioids such as morphine and hydromorphone with a large volume of distribution, rapid clearance, and short half-life (KuKanich et al., 2008b). The maximum concentration, 21.5 ng/ml, occurred at approximately 10 minutes, after 0.1 mg/kg SC with an elimination half-life of 1 hour. The pharmacokinetics in cats (Table 13.5) indicate that clearance is slower and half-life slightly longer than dogs (Pypendop et al., 2014).

Oxymorphone, 0.03 mg/kg IV, produced similar effects in horses as morphine. Although a veterinary formulation is FDA approved, it is not currently available. Intermittent availability problems with the human approved products have also occurred. Oxymorphone is not used commonly due to intermittent supply problems and cost. Current dose recommendations for oxymorphone are presented in Table 13.10.

Hydrocodone

Hydrocodone is a morphine derivative primarily used as an antitussive, but may also be effective for mild to

Table 13.10 Oxymorphone dosages (only parenteral doses are listed, oral administration is not recommended)

	IV	IV infusion	IM/SC
Dog	0.05–0.1 mg/kg q 2–4 h	0.005–0.01 mg/kg/h	0.05–0.1 mg/kg q 2–4 h
Cat	0.025–0.05 mg/kg q 2–3 h	0.01–0.02 mg/kg/h	0.025–0.11 mg/kg q 2–4 h
Horse	0.01–0.02 mg/kg once	NR	0.01–0.02 mg/kg once
Rat / mouse			0.2–0.5 mg/kg q 4 h
Guinea pig/ hamster			0.2–0.5 mg/kg q 4 h
Rabbit			0.2–0.5 mg/kg q 4 h
Primates			0.05–0.2 mg/kg q 4–6
Ferrets			0.05 mg/kg q 4–6 h

moderate pain. Hydrocodone appears to have the highest oral bioavailability of the opioids studied. The efficacy of hydrocodone is in part due to metabolism to hydromorphone, which occurs in dogs (Findlay et al., 1979; Barnhart and Caldwell, 1977). Demethylation of hydrocodone to hydromorphone is more prominent in dogs than the demethylation of codeine to morphine (Findlay et al., 1979). However, there appears to be variability in the metabolism of hydrocodone to hydromorphone with some studies reporting substantial amounts of hydromorphone produced (KuKanich and Spade, 2013; Findlay et al., 1979; Barnhart and Caldwell, 1977), whereas others report it is not a substantial metabolite (Benitez et al., 2015a; Li et al., 2013). Norhydrocodone has been identified as a metabolite in dogs after high doses (60–120 mg/day in unstated dog breed or weight) and it does have some μ opioid effects, about 70 times less potent than hydrocodone itself (Li et al., 2013; Navani and Yoburn, 2013). The absolute oral bioavailability of hydrocodone (34–44%) is also higher in dogs than codeine (6–7%) (Findlay et al., 1979). This may explain why many veterinarians prefer oral hydrocodone as an antitussive treatment over codeine in dogs.

Hydrocodone is available for oral use (syrup and tablets) as hydrocodone bitartrate. Preparations that contain hydrocodone for oral use in people in the United States may also contain homatropine (Hycodan) or acetaminophen and a recently approved version contains hydrocodone as a sole ingredient. Hydrocodone also is available with nonsteroidal antiinflammatory drugs (Table 13.11).

When administered as an antitussive, the homatropine is not expected to have therapeutic or adverse effects that affect coughing, but is included as an abuse deterrent. The recommended dose for dogs as an antitussive is 0.22 mg/kg of hydrocodone every 4–8 hours; for analgesia higher doses are likely to be needed, 0.44 mg/kg every 6–8 hours. A recent study reported the clinical efficacy of hydrocodone in combination with acetaminophen in postoperative orthopedic surgery dogs in which 15/25 were judged as having adequate analgesia using a subjective scale (Benitez et al., 2015b).

Table 13.11 Examples of opioids combined with nonsteroidal antiinflammatory drugs (NSAIDs)

Opioid	NSAID	Common brand name
Hydrocodone	Acetaminophen	Vicodin, Hydrocet, Lorcet
Codeine	Acetaminophen	Tylenol with Codeine
Codeine	Aspirin	Empirin
Hydrocodone	Aspirin	Damason
Oxycodone	Aspirin	Percondan
Oxycodone	Acetaminophen	Percocet, Tylox, Roxicet

Codeine

Codeine is a naturally occurring alkaloid similar in structure to morphine. Because of the availability of other opioids, parenteral codeine is rarely indicated for use in veterinary medicine, with codeine's use primarily limited to oral administration for mild pain most often in combination with acetaminophen (Table 13.11). The combination with acetaminophen is contraindicated in cats as acetaminophen is toxic.

There are multiple metabolites of codeine, for example the 6-glucuronide, norcodeine, and morphine. In people, approximately 10% of codeine is metabolized to morphine, via *O*-demethylation, but may be much lower than previously assumed (Shah and Mason, 1990). Morphine is further metabolized to other compounds in people, which may be active (i.e., morphine-6-glucuronide). It has always been assumed that the activity of codeine was produced by morphine or its metabolites, thus it would have approximately 10% of the efficacy of morphine. However, more recent evidence suggests that other metabolites (for example codeine-6-glucuronide and norcodeine) may contribute to codeine's analgesic activity (Lötsch et al., 2006). In dogs, the absolute oral bioavailability of codeine is low (4–7%) and morphine is a minimal metabolite, but there is substantial codeine glucuronidation in dogs (KuKanich, 2010; Findlay et al., 1979). The analgesic efficacy of oral codeine has not been examined in clinical trials with dogs or cats.

Oxycodone

Oxycodone is a morphine derivative with mild to moderate analgesic effects. In humans oxycodone has a 50–70% oral bioavailability and is available as an extended-release tablet (Table 13.11). Clinical use of oxycodone in animals has not been evaluated. Similar to the pattern observed for other opiates in dogs, oral systemic absorption is low. A dose of 0.5 mg/kg resulted in a C_{MAX} of only 6.5 ng/ml at 15 min and was below the limit of quantification (0.625 ng/ml) by 3 hours (Weinstein and Gaylord, 1979). However, only two dogs were evaluated. Noroxycodone was identified as a primary metabolite, but is 30 times less active than oxycodone. Oxymorphone was not assessed as a metabolite in dogs, but is a minor metabolite in humans (Söderberg et al., 2013). The low oral systemic availability would likely result in poor efficacy; therefore, its use is not currently recommended. It also has high abuse potential in people and would be risk if stocked in veterinary hospitals.

Heroin

Heroin is the diacetyl congener of morphine and is widely abused by humans. Heroin is available for medical use in some countries, but does not possess greater analgesic activity than morphine. The lipophilicity of heroin is greater than that of morphine resulting in a faster onset of action and the rapid euphoric effect following IV administration. Heroin is rapidly metabolized to morphine in most species. Although there is some historical use of heroin in veterinary medicine, current administration of heroin in veterinary medicine is not recommended due to the high abuse potential and it is listed as a Schedule I drug by the United States DEA (Table 13.2).

Methadone

Methadone has traditionally been used in humans to treat opiate addiction (Kosten and O'Connor, 2003). It was first shown to have this property in 1948 and was adopted as one of the principal treatments for opiate addiction in 1965. The advantage of methadone in this use is that the severity of withdrawal signs from methadone is much less than from heroin. It also has a much longer half-life in people (approximately 24 hours) than morphine or heroin and has high oral bioavailability. Therefore, addiction treatment programs can administer methadone once a day orally to avoid intravenous injections and achieve good compliance rates. At one time, methadone's only common use was for treatment of opiate addiction. However, it is now considered a valuable analgesic drug as well.

Methadone is approved for use in dogs by some countries. Methadone is a μ-receptor opiate agonist with high intrinsic activity. Methadone also has some activity as an antagonist at NMDA receptors and inhibits reuptake of norepinephrine and serotonin, all of which contribute variably to its analgesic activity. As a result, pain that is poorly controlled with other opioids (morphine, hydromorphone, and fentanyl), such as chronic and neuropathic pain, may be controlled more effectively with methadone (Foley, 2003). Activity on the NMDA receptor decreases tolerance from developing to the opiate effects. Additionally, methadone has demonstrated synergistic analgesic effects when coadministered with morphine and additive effects when administered in combination with oxymorphone, oxycodone, fentanyl, alfentanyl, or meperidine (Bolan et al., 2002). Methadone is a 50 : 50 racemic mixture, with the L-chiral isomer (L-enantiomer) primarily responsible for the opioid pharmacological effect and both isomers, L- and D-methadone, capable of binding to the NMDA receptor. Levomethadone contains only the L-enantiomer, therefore the administered dose should be half of the racemic formulation. Although oral formulations of methadone are inexpensive and readily available, the oral bioavailability is low and produced inconsistent plasma concentrations after oral administration to dogs (KuKanich and KuKanich, 2015; KuKanich et al., 2011, 2005d). Although not specifically studied in cats, the oral bioavailability may be higher in cats as the clearance suggests it is not a high hepatic extraction ratio drug (Pypendop et al., 2014) (Table 13.5).

Methadone appears to be well tolerated in dogs following IV administration, with adverse effects routinely occurring with morphine such as nausea, vomiting, defecation, and dysphoria not reported with methadone. Sedation was a prominent adverse effect and, as with other μ-receptor agonists, dose-dependent respiratory depression occurs, which is typically clinically insignificant with appropriate dosages (KuKanich et al., 2005d; KuKanich and Borum, 2008b; Garafolo et al., 2012). Cardiovascular effects are noted in healthy dogs following clinically relevant methadone dosages (0.5–1 mg/kg IV), with decreased cardiac output (~20%) and increased systemic vascular resistance (~50–100%) (Maiante et al., 2009; Stanley et al., 1980). The methadone LD_{50} in dogs (29 mg/kg IV) is proportionally lower relative to the clinical dose (0.5 mg/kg IV) than morphine (LD_{50} = 175 mg/kg IV, clinical dose = 0.5 mg/kg IV), but the LD_{50} is still 60 times higher than the clinical dose (Kasé et al., 1959). Thus, methadone has a large safety margin in healthy dogs, but should be administered cautiously in animals prone to congestive heart failure, with underlying cardiac disease, or hypertension.

The analgesic potency of methadone is similar to that of morphine (Table 13.8) and as a result the administered dose range is similar. However, the elimination of methadone is slower, resulting in longer dosing intervals as compared to morphine. The recommended dose of methadone for dogs is 0.5 mg/kg IV every 4–6 hours or 0.5–1 mg/kg every 6–8 hours IV, IM, SC. A study of methadone in Greyhounds indicated methadone is rapidly eliminated after IV administration in this breed with a higher dose and more frequent administration recommended 1 mg/kg q 3–4 hours (KuKanich and Borum, 2008b). Repeated IM and SC injections may result in tissue irritation and inflammation.

In cats, methadone, 0.6 mg/kg IM, was well tolerated when administered as a preanesthetic (Bley et al., 2004) and was effective for postoperative pain at a dose of 0.5–0.6 mg/kg (Dobromylskyj, 1993; Bley et al., 2004). Although repeated doses were not evaluated, a suggested dose interval based on pharmacokinetics and clinical observations is every 6–8 hours in cats. At a dose of 0.2 mg/kg SC to healthy cats, there were no adverse effects observed, euphoria was exhibited, and it was as effective against nociceptive stimuli as morphine (Steagall et al., 2006). At higher doses of 0.6 mg/kg SC, in the perioperative setting, it provided better analgesia than butorphanol in cats and was well tolerated (Warne et al., 2013). Administered via the oral transmucosal route to cats at a dose of 0.6 mg/kg, it was well tolerated and provided antinociceptive effects similar to the intravenous route (Ferreira et al., 2011).

Methadone appears to be well tolerated in horses when administered up to 0.2 mg/kg IV in combination with an α-2 agonist (Schatzman et al., 2001; Nilsfors et al., 1988). It was administered to horses at a dose of 0.15 mg/kg by the oral route, intragastric route, and IV and was well tolerated at concentrations that are similar to those considered analgesic for people (Linardi et al., 2012). No behavioral effects such as excitement, sedation, increased locomotor activity, or decrease gastrointestinal tract activity associated with other opioids were observed with methadone. The half-life was approximately 1 hour with a moderate volume of distribution. When methadone was administered through a nasogastric tube oral absorption was low (30%), but the absorption was much higher from administration directly into the mouth, suggesting oral transmucosal absorption occurred (Linardi et al., 2012). It also has been used in horses with ketamine protocols and as an anesthetic adjunct. Minimal cardiovascular effects were observed and as expected there was an increase in sedation with the combination as compared to the α-2 agonist alone. Healthy, pain-free horses administered methadone (0.12 mg/kg IV) as a sole agent showed increases in heart rate, arterial blood pressure, and cardiac output, but only minimal effects on blood gas parameters. Euphoria and dysphoria was of approximately 15 minutes in duration, but was subjectively less than the same effects produced by morphine, meperidine, and oxymorphone, but similar to that of pentazocine (Muir et al., 1978). An advantage of methadone in horses is that at doses in the range of 0.1 mg/kg, IV, sedation occurs, but they did not become recumbent. The horses maintained a sedated standing position without incoordination, allowing minor procedures to be performed.

Methadone is available as an oral flavored concentrate 1–10 mg/ml, as tablets for suspension (40 mg), and as oral tablets (5, 10 mg). It is also available as an injectable solution (10 mg/ml).

Meperidine (Pethidine)

The availability of newer opioids has resulted in a dramatic decrease in the use of meperidine in veterinary medicine. Meperidine (meperidine is the USAN terminology, but pethidine is used in the BAN) is a synthetic opioid with antimuscarinic and negative inotropic effects that are different from most of the other opioids. Meperidine appears to have the most cardiac depressant effects of the opioids. Similar to morphine, histamine release occurs following IV administration, but the clinical relevance appears to be minimal as adverse effects were not reported following intravenous meperidine administered to dogs (2 and 5 mg/kg) or cats (11 mg/kg) (Ritschel et al., 1987; Davis and Donnelly, 1968). Meperidine is less potent than morphine, therefore higher doses are needed (Table 13.8). It may be less constipating and cause less nausea than morphine. A meperidine metabolite, normeperidine, has CNS excitatory effects and accumulates following multiple doses in humans and can result in seizures. This metabolite in people has contributed to the *serotonin syndrome* that is associated with some drugs and drug combinations and is a precaution to its use in human medicine today. However, normeperidine appears to be a minor metabolite in dogs and cats. Meperidine as the parent drug can also exhibit some effects on serotonin receptors and can result in serotonin syndrome if combined with a monoamine oxidase inhibitor (selegiline), tricyclic antidepressant, serotonin reuptake inhibitor, or tramadol. Pharmacokinetics are summarized in Table 13.5. Meperidine is well absorbed following IM administration to dogs and cats, but the rate of absorption is variable. Meperidine has poor oral and transmucosal bioavailability in dogs.

Meperidine (1.1 mg/kg IV) produces similar adverse effects in horses as morphine (0.1 mg/kg). The duration of analgesia is approximately 1 hour as meperidine is rapidly redistributed following IV administration. The elimination half-life is 1 hour and multiple doses would be expected to result in drug accumulation, longer duration of effect, and increase the risk of altered gastrointestinal tract motility.

Fentanyl

Fentanyl is a synthetic opioid that is selective for the μ-opioid receptor. Its use has increased in veterinary medicine as an intravenous drug and transdermal preparation. Similar to other opioids, fentanyl has minimal cardiovascular effects at clinically relevant dosages in healthy animals. There is a dose-dependent respiratory depression, but this is not a clinical problem in healthy veterinary species (Grimm et al., 2005; Hug and Murphy, 1979). Fentanyl has a wide safety profile with doses as high as 300 times the recommended dose not being lethal in spontaneously breathing dogs (Bailey et al., 1987). Even when high-dose fentanyl transdermal delivery was administered to dogs undergoing surgery, fentanyl did not produce postoperative respiratory depression (Welch et al., 2002). It is highly lipophilic – more than 1,000 times more lipophilic than morphine (Table 13.6). As a result, the onset of action is rapid in comparison to the other opioids because of rapid diffusion into the CNS. Fentanyl CSF concentrations peak between 2.5 and 10 minutes following IV administration, in comparison to morphine which peak between 15 and 30 minutes in dogs. Due to the rapid penetration into the CNS, effects on the respiratory center occurs immediately following IV fentanyl

Table 13.12 Fentanyl dosages

	IV	IV infusion	IM/SC
Dog	5–10 µg/kg q 1–3 h	2–5 µg/kg/h	5–15 µg/kg q 1–3 h
Cat	5–10 µg/kg q 1–3 h	2–5 µg/kg/h	5–10 µg/kg q 1–3 h
Horse	2–4 µg/kg q 3–4 h	0.3–0.5 µg/kg/h	2–4 µg/kg q 3–4 h

Note: Dosages are in µg/kg. 1000 µg = 1 mg.

administration. Fentanyl is primarily eliminated by metabolism, with hydroxylation and dealkylation being the primary mechanisms, and less than 8% of the total dose eliminated as unchanged drug. Pharmacokinetics are summarized in Table 13.5 and current dose recommendations for fentanyl are presented in Table 13.12.

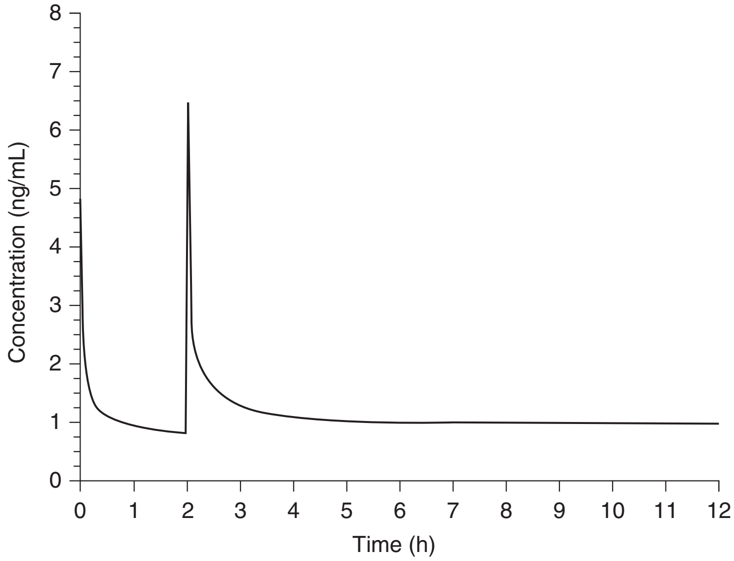
As a very lipophilic molecule with high potency (Table 13.8), fentanyl is ideally suited for transdermal penetration to produce systemic effects. A veterinary approved fentanyl transdermal solution is available for use in dogs and a transdermal delivery device, generally referred to as the "fentanyl patch," which bypasses first-pass hepatic metabolism resulting in therapeutic plasma concentrations, is used in an extralabel manner in cats and dogs.

Dogs

Fentanyl is well tolerated in dogs with a lower occurrence of nausea, vomiting, and dysphoria as compared to some of the other opioids, but variable sedation has been observed. In dogs fentanyl is about 60% bound to plasma proteins and protein binding is linear with plasma concentration. Pharmacokinetics are listed in Table 13.5. Following IV bolus administration, plasma fentanyl concentrations decrease rapidly, primarily due to redistribution with a concurrent rapid decrease in the effects. The distribution half-life is approximately 45 minutes (Kyles et al., 1996). This phenomenon is similar to that seen with thiobarbiturates where redistribution from the CNS is primarily responsible for recovery from anesthesia. There is a more prolonged terminal phase with a mean elimination half-life in dogs of up to 6 hours (KuKanich and Allen, 2014; Kyles et al., 1996), indicating steady-state plasma concentrations will be achieved at 18–24 hours if administered as an IV infusion. In order to rapidly achieve and maintain targeted plasma concentrations, a loading dose of fentanyl is recommended at the beginning of an IV infusion and may need to be repeated, especially in severely painful animals (Figure 13.2). Subcutaneous fentanyl is rapidly absorbed with plasma concentrations exceeding 0.5 ng/ml persisting for about 4 hours after 15 µg/kg SC (KuKanich, 2011). Pain occurs on SC injection because the formulation of fentanyl citrate can be irritating to tissues, but addition of sodium bicarbonate (8.4%) at a ratio of 1 part sodium chloride to 20 parts fentanyl injection eliminated the pain associated with the injections.

Transdermal fentanyl solution (Recuvyra®) is approved for use in dogs with expected analgesic effects occurring within 2–4 hours and persisting for about 96 hours. However, there can be a variation in response because of interanimal variability in the extent of absorption and duration of effects. A risk minimization action plan (RiskMAP) is required for safe and appropriate use of the transdermal solution since it is highly concentrated (50 mg/ml compared to 0.05 mg/ml for the conventional injection of fentanyl citrate). It is absorbed through intact skin and human exposure could result in serious and potential life-threatening respiratory depression. Specific requirements must be met for its application: two trained people are required for administration, a specific applicator and syringe are

Figure 13.2 The expected plasma profile of fentanyl administered as two loading doses, 5 µg/kg IV, at time 0 and 2 h and IV infusion 2 µg/kg/h starting at time 0 in dogs. The second IV loading dose is administered to prevent drug concentrations from dropping below 1 ng/ml. The targeted plasma concentration of fentanyl is 1 ng/ml for analgesia.



required for administration, protective clothing (lab coat, safety glasses, and impermeable gloves) are required, the solution should only be applied in a hospital setting, animals treated with the transdermal solution should be gently restrained for 5 minutes to allow the solution to dry, and hospital staff should wear impermeable glove when handling the treated dogs. Due to the variability in drug absorption a small percentage of treated dogs may need rescue analgesia and some dogs may have profound opioid effects in which naloxone is effective at reversing.

Fentanyl is well absorbed, but variably, in dogs when administered by a transdermal patch (Kyles et al., 1996) with patches requiring changing every 72 hours (3 days) to maintain a therapeutic effect. There is a 12 to 24-hour lag time before therapeutic effects are achieved following patch application, consistent with the time needed to achieve steady state from an IV infusion, as noted above, resulting in effective concentrations for 2 day, from 24 to 72 hours. Although the fentanyl patches are not approved for animals, the human sizes are used. Fentanyl patches are available in sizes of 12.5, 25, 50, 75, and 100 μg/h delivery rates. Despite this range of sizes, there have been no differences observed in plasma concentrations in dogs when 50-μg/h patches were compared with 100-μg/h patches (Egger et al. 1998). High-dose patches were well tolerated in dogs (Welch et al., 2002) and have been effective for treating both soft-tissue and orthopedic surgical pain in dogs (Robinson et al., 1999; Kyles et al., 1998). Fentanyl patches should be prescribed cautiously as diversion, abuse, and accidental exposure could occur when used for outpatients. People have died after leaving the hospital because they were sensitive to fentanyl and developed respiratory depression. Death in children have been reported due to exposure to fentanyl transdermal patches. Despite the evidence for absorption of fentanyl from a patch or the transdermal solution, fentanyl formulated in a pluronic lecithin organogel (PLO) transdermal gel failed to reach 1 ng/ml, indicating it is not an effective formulation in dogs (Krotscheck et al., 2004).

Cats

Cats have tolerated fentanyl well with only mild adverse effects that have included sedation, purring, rubbing, kneading, and mydriasis (Robertson et al., 2005a). The total body clearance of fentanyl is similar to that of dogs, but because of a much lower volume of distribution, the elimination half-life is much shorter (~2.5 hours) (Lee et al., 2000). The fentanyl transdermal solution approved for dogs should not be administered to cats because there are differences in drug absorption. Fentanyl is also well absorbed, but variably, in cats from the transdermal patch (Lee et al., 2000). Doses most often used for fentanyl patches in cats are the 25-μg/h patch. In very small cats, a 12.5-μg/h patch can be used or one-half of a 25-μg/h patch can be covered (absorption is dependent on the exposed surface area of the patch) resulting in a rate of delivery reduced by approximately one-half (Davidson et al., 2004).

Because of the shorter half-life in cats, there is a 6 to 12-hour lag time to therapeutic effect following application of fentanyl patches, which is shorter than in dogs. In contrast to dogs, the drug concentrations persist longer in the skin of cats, resulting in a longer terminal half-life after fentanyl patches are removed (Lee et al. 2000). Therapeutic plasma concentrations can be maintained for 120 hours (5 days) in cats after administration of a 25-μg/h patch. Clinical studies have demonstrated efficacy of transdermal fentanyl in cats for pain from surgical onychectomy (Gellasch et al., 2002; Franks et al., 2000).

Horses

Although fentanyl has been administered IV in some analgesic and anesthetic protocols for horses, it is not used as often as other opioids. When fentanyl is administered IV to horses, it is relatively well tolerated, but stimulation of locomotor activity has been well documented at 20 μg/kg (Mama et al., 1992) and at even lower doses of 5 μg/kg (Kamerling et al. 1985).

The use of fentanyl transdermal delivery is not as practical in horses as it has been in dogs and cats. Fentanyl is well absorbed following application of transdermal patches in horse (Maxwell et al., 2003), but concentrations are variable and short lived. When two 100-μg/h fentanyl patches were applied to horses, there was high absorption and plasma concentrations that were in a range considered to be therapeutic, but not as long lasting as in small animals. In another study, after application of three 100-μg/h patches to horses, the concentrations were in a therapeutic range, but were variable (Orsini et al., 2006). There was a peak at approximately 12 hours, but thereafter it declined during the next 72 hours without attaining a steady-state plateau as seen in the smaller animals.

Application of fentanyl patches to horses for controlling pain has produced mixed results. Low doses delivered by one or two 100-μg/h patches have not produced consistently effective results. Fentanyl was capable of controlling visceral pain in horses without producing adverse effects, but that somatic musculoskeletal pain was less effectively controlled, or may require higher doses (Orsini et al., 2006). Thomasy et al. (2004) found that transdermal fentanyl controlled musculoskeletal pain in some horses that had not responded to nonsteroidal antiinflammatory drugs (NSAIDs) alone, but improvement was minimal. In the study by Orsini et al. (2006) they concluded that a minimum of three 100-μg/h patches are necessary to control pain in horses, and some horses may not respond. To maintain effective concentrations, patches should be reapplied every 36 to 48 hours, in contrast to dogs, cats, and people. Alternatively, the authors propose (Orsini et al., 2006) that patches should not be removed for 100 hours but new ones applied during treatment at 36 hours and every 24 hours thereafter.

Other Large Animal Species

Fentanyl also has been investigated as an anesthetic adjunct and analgesic in sheep, goats, and llamas. In the study by Grubb et al. (2005) the application of four 75-μg/h fentanyl patches produced plasma fentanyl concentrations judged to be in a therapeutic range and it was well tolerated.

Fentanyl was administered IV and via transdermal patch to goats (Carroll et al., 1999). Although the IV half-life was short (1.2 hour mean), it was otherwise well tolerated. A single 50-μg/h transdermal patch produced plasma concentrations in an effective range, but the results were highly variable among goats. Plasma concentrations were not sustained at a steady-state level that has been observed in other animals.

Sufentanil, Alfentanil, Remifentanil

Sufentanil, alfentanil, and remifentanil are fentanyl derivatives that exhibit similar pharmacodynamic effects as fentanyl. Sufentanil is five to seven times more potent than fentanyl and eliminated metabolically similar to fentanyl. Alfentanil is a fentanyl derivative that is less potent, but eliminated in a similar manner. Remifentanil is unique among the fentanyl derivatives as it undergoes nonhepatic metabolism due to esterases in the tissues (primarily muscle and intestinal) and plasma resulting in a short terminal half-life of only 3–6 minutes and a rapid clearance in dogs. Clinical use of remifentanil is uncommon but it can be used during induction anesthetic protocols and administered with a constant rate infusion. Because of the short half-life, steady-state concentrations are achieved rapidly. It can be administered with other drugs, including inhalant anesthetics, propofol, α_2-agonists, sedatives, and tranquilizers. Because it does not require hepatic metabolism or renal elimination, it can be administered safely to patients that have liver or kidney disease. Remifentanil use in dogs is limited to a few studies and case reports. Doses in animals have been extrapolated from humans (human starting dose is 0.1 μg/kg/min constant rate infusion). Remifentanil has been infused safely in cats, and over a wide range of doses (0.06–16 μg/kg/min) IV it did not affect isoflurane minimum alveolar concentration (MAC).

Carfentanil and Etorphine

Carfentanil and etorphine are opioid agonists primarily used for sedation and capture of zoo and wild animals. For a complete discussion of the use of these drugs for this indication, the reader is referred to more detailed book chapters (Carpenter and Brunson, 2007; Caulkett and Arnemo, 2007). Carfentanil is a very potent fentanyl derivative used primarily for immobilization of zoo and wildlife species. It is reported to be 8,000–10,000 times more potent than morphine. Carfentanil is a DEA schedule II controlled substance with additional registration and storage requirements, which must be met prior to obtaining it. The capture dose is 0.005–0.02 mg/kg and carfentanil is available as a 3-mg/ml solution. The high potency of carfentanil allows specialists in this field to administer small volumes via projectile dart.

Etorphine (M99) has a potency of as much as 3,000–4,000 times that of morphine. The most common use of etorphine has been to capture wild animals and restrain zoo animals. Like carfentanil, the high potency allow it to be used in the field by administration via small volumes in a projectile dart. As little as 8–10 mg or less (total dose) can bring down a full-grown African elephant. It is usually reversed with the antagonist, diprenorphine (M 50–50). Because of the high potency of etorphine, it should only be used by trained and experienced individuals who are aware of the risks.

After IM injection, the action of etorphine is prompt, within 20 minutes. The animals may then be easily restrained or brought to lateral recumbency. If the action is not antagonized, the immobilized state usually persists from 30 to 60 minutes. Used by itself in exotic species, the IM doses of etorphine that usually result in rapid immobilization, sedation, and analgesia are listed in Table 13.13. For most hoofed zoo animals, the dose is approximately 1–2 mg (total dose). For example, a zebra requires about 1.5 mg and the rhinoceros 1–1.5 mg (total dose).

For some animals, etorphine has been combined with the α_2-agonist xylazine. Hyaluronidase (150 IU) has also been added to etorphine and etorphine/xylazine mixtures to increase the absorption rate.

Etorphine or carfentanil should not be used in animals intended for food. There are restrictions on using such immobilizing agents near the hunting seasons. Currently, the only opioids marketed in the United States for use in food-producing species are carfentanil and naltrexone, which are labeled for use in Cervidae. Carfentanil and naltrexone should not be administered within 30 and 45 days, respectively, of a hunting season.

Table 13.13 Doses of etorphine in animals for immobilization

Family	Dose (mg/45 kg or 100 pounds)
Equidae (Mongolian horse, zebra)	0.44
Ursidae (black, grizzly, polar bear)	0.5
Cervidae (fallow deer, moose)	0.98
Bovidae (antelope, bighorn sheep)	0.09

Safe use of etorphine and carfentanil require special precautions. Carfentanil or etorphine should never be used without an antagonist available (such as naltrexone) or be handled without training and experience. These are lipophilic drugs and there is the potential for exposure to lethal doses from skin contact and transdermal absorption. The lethal dose of etorphine for adult humans is small – only 30–120 μg (0.03–0.12 mg) total dose; therefore, accidental injection requires prompt emergency treatment.

Accidental injections of small amounts of etorphine–acepromazine have led to serious respiratory depression, coma, and death in people. In the event of an accidental injection of etorphine, administration of naloxone (0.8 mg) is necessary, to be repeated at 5-minute intervals if symptoms are not reversed. Naloxone, rather than diprenorphine should be used for reversal.

Propoxyphene

Propoxyphene is a methadone congener, which is considered to possess less analgesic activity than codeine but can cause marked respiratory depression. Due to the low efficacy of propoxyphene its use in veterinary medicine is not recommended.

Apomorphine

Apomorphine is a morphine degradation product, but its primary pharmacological effects are not due to the opioid receptor system. Apomorphine primarily acts as a dopamine (D2) agonist. The dopaminergic effects have been used to treat neurodegenerative diseases in people. In Parkinson's disease there is a deficiency of dopamine, and apomorphine's dopaminergic effects can be helpful. A sublingual formulation is also used to treat erectile dysfunction in men.

Through its dopaminergic effects, apomorphine will reliably induce vomiting in dogs. It is used to treat poisoning in dogs if administered shortly after a dog has ingested a poison. It is not as effective as an emetic in cats, which illustrates the interspecies differences in distribution and response of opiate receptors. (In cats, xylazine, an α_2-agonist, is a more reliable emetic.)

Apomorphine's action is presumably via direct stimulation of the chemoreceptor trigger zone (CRTZ) in the area postrema (Mitchelson, 1992). Alternatively, it acts as a dopamine (D_2) agonist, which is a neurotransmitter in both the CRTZ and vomiting center. At high concentrations it acts as an *antiemetic,* presumably via action on central opiate μ-receptors (Scherkl et al., 1990). Naloxone increases, rather than decrease, the emetic effects because naloxone blocks the μ-receptor-mediated antiemetic effects. The route of administration can also influence the effect. Rapid increases in blood concentrations, such as from IV or rapid IM absorption, can produce antiemetic effects by opposing the emetic action in the CRTZ. This explains why lower concentrations produced by SC and mucosal administration may be more effective for producing emesis.

The dose used in dogs has covered a wide range, with the ED_{95} reported to be 0.02 mg/kg IV to 4 mg/kg orally. The most reliable doses listed in review papers are 0.1 mg/kg SC or 0.05 mg/kg IV. After IV injection of 0.03–0.1 mg/kg it produces vomiting reliably in dogs. However, it was more effective if administered SC than if given IM, probably because of rates of absorption as mentioned above (Scherkl et al., 1990). In these studies, doses of 0.04 and 0.1 mg/kg were studied. Alternatively, a 6-mg tablet of apomorphine hydrochloride can be mixed with sterile water or saline solution. A few drops of this solution can be placed in the conjunctival sac. When vomiting occurs, the solution should be promptly rinsed out of the eye. The pH of this hydrochloride solution is low, so it can be irritating to the ocular membranes if not rinsed. Emesis was successfully induced with apomorphine in 100% of dogs at a dose of 0.03 mg/kg IV once, or in 91% of dogs when a crushed tablet dissolved in saline solution was administered to the conjunctival sac (Khan et al., 2012).

The half-life of apomorphine in dogs is 48 minutes after IV injection and it is highly absorbed from IM and SC injection (Scherkl et al., 1990). Apomorphine is much less active orally because of high first-pass metabolism. Adverse effects are attributed to the dopaminergic effects (e.g., hypotension, drowsiness, dyskinesia).

Apomorphine hydrochloride can be obtained from some sources as a 6-mg tablet, or a 2-ml ampule (Apokyn) at a concentration of 10 mg/ml for IV, SC, or IM use. For people, a 3-ml cartridge (10 mg/ml, Apokyn) injectable formulation is administered SC – usually 2 to 6 mg per injection – to treat episodes of severe Parkinson's disease (to be coadministered with an antiemetic) (Lees, 1993).

Partial and Mixed Receptor Opioids

Buprenorphine

Buprenorphine is a high-affinity partial agonist at the μ-opioid receptor, but an antagonist for the κ-receptor (Johnson et al., 2005). Its effects on analgesia, sedation, euphoria, gastrointestinal effects, and respiratory depression are attributed to the μ-opioid receptor activity. Buprenorphine exhibits a ceiling to its analgesic and respiratory depressant effects, in which increased doses do not produce a greater increase in response. It also appears to have a higher safety profile than full μ-receptor agonists. For example the ratio of LD_{50} to ED_{50} – a measure of the therapeutic index – is 464 for morphine, but up to 12,313 for buprenorphine. The LD_{50} in dogs is 79 mg/kg, but therapeutic doses usually do not exceed 0.04 mg/kg. In cats, compared to full agonist μ-receptor agonists such as morphine and hydromorphone, it produces less dysphoria, excitement, vomiting, or nausea. It is a Schedule III narcotic (Table 13.2) and has less potential for abuse than pure μ-opioid receptor agonists. There are formulations available for use in people that are used for treatment of opiate addiction.

The cardiovascular safety of buprenorphine has been evaluated in dogs and horses (Carregaro et al., 2006; Martinez et al., 1997). The changes observed were not important enough to produce clinical significance.

Because buprenorphine is a μ-opioid receptor partial agonist, and a κ-receptor antagonist, there is concern that it may inhibit the effects of coadministered opioids. Although theoretically it is possible that buprenorphine could antagonize the antinociceptive effects of other opioids, there is no clinical evidence of this property. In laboratory animals, preadministration of buprenorphine did not cause impairment of μ-receptor acceptability beyond the duration of antinociceptive activity (Englberger et al., 2006). Nevertheless, buprenorphine binds to the μ-opioid receptor with higher affinity than other opioids. It will displace other opioids to elicit a withdrawal response in individuals with opioid dependence. Because of the high receptor affinity, it will require a much higher dose of naloxone to reverse respiratory depression and other opioid effects compared to doses used to reverse effects from morphine.

The high affinity of buprenorphine for the μ-opiate receptor may induce a longer duration of pain control in animals. This duration of analgesia in cats has been reported to be less than 4 hours (Taylor and Houlton, 1984; Robertson et al., 2005b; Stanway et al., 2002), up to 5 hours (Johnson et al., 2007), and perhaps as long as 8 hours. In dogs, the analgesic effects were not much longer than the effects from morphine (Brodbelt et al., 1997). The potency of buprenorphine is approximately 25 times that of morphine (Table 13.8). In some of the clinical studies of buprenorphine in humans it had comparable efficacy to that of morphine. In animals, the efficacy of buprenorphine has been evaluated in some veterinary studies of surgical pain – primarily in cats. It was more effective than morphine in cats (Stanway et al., 2002), more effective than oxymorphone or ketoprofen (Dobbins et al., 2002), and equally effective as butorphanol (Johnson et al., 2007). When administered subcutaneously in cats (0.02 mg/kg) it produced a minimum antinociceptive effect in comparison to morphine or methadone, but the route of administration may have limited its effect (Steagall et al., 2006). It was equally effective as morphine in dogs in postoperative orthopedic surgery (Taylor and Houlton, 1984) and similarly as effective as transdermal fentanyl solution in postoperative soft tissue and orthopedic dogs (Linton et al., 2012). It is, at minimum, effective for treatment of mild to moderate pain and perhaps effective for more severe pain. In most of the studies in which the analgesic effects have been evaluated, a range of doses of 10 μg/kg (0.01 mg/kg) to 40 μg/kg have been used in dogs, cats, and horses, which is higher than doses used in people (4–8 μg/kg). However, as stated previously, results of clinical and research studies have varied.

The oral bioavailability of buprenorphine is low because of rapid clearance and first-pass metabolism. Buprenorphine has high lipophilicity (Table 13.6) and lipophilicity is increased at a pH higher than its pKa of 8.3. It has a large volume of distribution (greater than 7 l/kg) and high brain to plasma tissue ratios. Buprenorphine is highly protein bound in whole blood. In dogs, buprenorphine glucuronide conjugates appear to be the primary metabolites, which are eliminated via biliary secretion. Studies suggest some of the glucuronide conjugates of buprenorphine and norbuprenorphine may provide analgesic effects (Brown et al., 2011). The metabolism of buprenorphine in cats has not been reported.

Similar to other opioids, clinically relevant cardiovascular changes are minimal but increases in total peripheral resistance can occur. Buprenorphine is well tolerated in dogs and cats with sedation, mydriasis (cats), and euphoria occurring as adverse effects. Nausea and vomiting are possible, but occur rarely with buprenorphine. Excitement in horses after doses of 5 and 10 μg/kg IV have been observed (Carregaro et al., 2007), which was characterized as head-nodding, shifting, trotting, and restlessness. Excitement may be less in horses that have clinical pain.

Despite the lipophilicity of buprenorphine the onset of effect appears to be delayed following IV administration. Initially the lag in effect was thought to be due to slow binding to the opiate receptors, but studies in rats indicate the distribution into the CNS may be the rate-limiting step (Yassen et al., 2005). The effect of buprenorphine may also last longer than the plasma concentrations because of the slow diffusion out of the CNS and high affinity for the μ-opioid receptors.

The oral mucosal (buccal, sublingual) administration had high bioavailability (>100%) in one study (Robertson et al., 2003, 2005b), but follow-up studies showed that this value may have been biased. Lower absorption of approximately 30% was shown by Hedges et al. (2014) and they attributed this difference to the method of blood collection. When blood samples were collected from the jugular vein, it artificially increased the concentrations measured because of the anatomic proximity of the jugular vein to the oral mucosal venous drainage. The lower absorption from transmucosal administration to cats was also apparent in the analgesic response as measured by thermal threshold (Hedges et al., 2014). Cats administered oral mucosal buprenorphine (20 μg/kg) had increases in thermal threshold, but it was transient and of limited magnitude compared to the IV dose.

Oral mucosal absorption in dogs has been reported to be 38%, but this also may be overestimated as blood samples were obtained from the jugular vein, which directly drains from the site of administration, and, clinically, rescue analgesia was more frequent and was needed sooner unless a high (120 μg/kg) transmucosal dose was administered (Abbo et al., 2008; Ko et al., 2011). The oral transmucosal bioavailability is low in horses and the bias of jugular sampling overestimating buprenorphine plasma concentrations was documented in horses, as in cats (Messenger et al., 2011). In humans, there are sublingual tablets used primarily for treatment of opiate addiction. The sublingual absorption in humans is approximately 30%.

Buprenorphine has been extensively studied in cats. Steagall et al. (2014) reviewed these studies and readers are referred to that paper for greater detail. Important conclusions from Steagall et al. (2014) can be summarized here. Buprenorphine has a delayed onset of action in cats, and exerts moderate analgesic effects, with the duration of action shorter than suggested in other papers (e.g., 4-hour duration rather than 8-hour duration). Low doses of 20 μg/kg subcutaneously are not recommended because of low absorption and poor efficacy. The IV or IM dose is more effective and doses of 20 μg/kg or higher are recommended. The oral mucosal dose should be increased (40 μg/kg) to overcome low bioavailability. Overall, the clinical trials have demonstrated that analgesic effects in cats are inconsistent and difficult to compare because of different methods of pain evaluation, surgical procedures, dose, and route of administration. Although it is often assumed to be effective, these authors concluded that effects are inconsistent and "buprenorphine may not provide sufficient analgesia in some cats". It is also observed that the only veterinary approved product for cats (Simbadol^{TM}, 1.8 mg/ml) is administered to cats at a dose of 0.24 mg/kg SC (240 μg/kg) once daily. This dose was shown to be safe and effective in clinical trials, but it is much higher dose than used in earlier studies.

Oral (per os) bioavailability of buprenorphine is low (9.7%) and variable in rats, and less than 10% in people. In rats it was not effective by oral administration until high dosages (≥5 mg/kg PO) are administered (Martin et al., 2001; Brewster et al., 1981). The dose resulting in analgesia (5 mg/kg) was not voluntarily eaten in gelatin by the rats and needed to be administered via gavage.

Transdermal absorption of buprenorphine has been reported in dogs after application of the patch approved in Europe. A slow absorption occurred with a mean T_{MAX} of 55 hours and C_{MAX} of 2 ng/ml after applying a 52.5-μg/h patch to 12.7 kg Beagles (Pieper et al., 2011). In cats weighing 5.1–7.4 kg, a 35-μg/h transdermal patch resulted in a slow increase in plasma buprenorphine concentration until the patch was removed at 72 hours, with a mean buprenorphine concentration of 4.24 ng/ml (Murrell et al., 2007). The pharmacokinetics of transdermal buprenorphine patches have not been reported in horses.

Buprenorphine hydrochloride is available in Europe as a veterinary formulation (Vetergesic). In the USA, the human formulation is commonly used (Buprenex, 0.3 mg/ml) and a formulation is specifically approved for cats in a once-daily subcutaneous formulation (Simbadol, 1.8 mg/ml). There are numerous other formulations approved for human use including sublingual tablets, transdermal patches, buccal film, and sublingual films to treat opiate addiction. Dose recommendations for buprenorphine use in animals are presented in Table 13.14.

Table 13.14 Buprenorphine dosages

	IV / IM / SC	Oral transmucosal
Dog	0.01–0.04 mg/kg q 4–8 h	0.12 mg/kg q 8 h (low absorption and impractical; therefore not recommended)
Cat	0.01–0.02 mg/kg q 4–8 h (SC not recommended at these doses) 0.24 mg/kg SC, q 24 h, for 3 days (use 1.8 mg/ml Simbadol formulation).	0.02–0.04 mg/kg q 4–12 h
Horse	0.005–0.01 mg/kg	NR
Rat / mouse	0.05–0.1 mg/kg q 4–12 h	NR
Guinea pig / hamster	0.05–0.1 mg/kg 4–12 h	NR
Rabbit	0.01–0.05 mg/kg q 4–12 h	NR
Primates	0.005–0.01 mg/kg q 4–12 h	NR
Ferrets	0.01–0.03 mg/kg q 4–12 h	NR

NR, not recommended.

Butorphanol

Butorphanol is a κ-opiate receptor agonist and either a partial agonist or antagonist at the μ–opiate receptor depending on the study. The potency is often reported to be five to seven times that of morphine, but unpublished data by the author (BK) indicates butorphanol is closer to equipotent with morphine in dogs (Table 13.8). The efficacy of butorphanol is dose dependent, but plateaus with efficacy limited to mild and moderate pain. Butorphanol is a commonly used analgesics and anesthetic adjuncts in veterinary medicine.

Butorphanol is a weak base with a pKa of 10.7, and is highly lipophilic with a Log P (octanol : water) of 3.3 (Table 13.6). Preparations of butorphanol are available commercially as butorphanol tartrate. One mg of butorphanol tartrate is equivalent to 0.68 mg of butorphanol base. The commercially available injectable preparation (Torbugesic, Torbutrol, Stadol) is available in 0.5, 2, and 10 mg of butorphanol (base)/ml in a buffered solution (pH of 3.5 to 5.5). Tablets (Torbutrol) are available as 1, 5, and 10 mg of butorphanol (base).

Butorphanol has been commonly used in cats with a dose range of 0.1 to 0.8 mg/kg. Above 0.8 mg/kg there is a ceiling effect and no further beneficial effects occur with higher doses. The duration of effect is reportedly not increased with higher doses (Sawyer et al., 1991; Lascelles and Robertson, 2004). Routes of administration include intramuscular, intravenous, subcutaneous, and oral. Pharmacokinetics are listed in Table 13.5. The comparison of clearance and terminal half-life between dogs and cats indicates substantially slower elimination in cats compared to dogs.

Compared to less lipophilic opioids such as hydromorphone, butorphanol is reported to have a faster onset of effect (Lascelles and Robertson, 2004), although in other studies the onset was longer (Johnson et al., 2007). The duration of antinociceptive activity of butorphanol is variable, depending on the report. It has generally been 3 hours or less in most studies (Lascelles and Robertson, 2004; Sawyer and Rech, 1987), and from less than an hour to 1.5 hours in dogs (Grimm et al., 2000; Sawyer et al., 1991). In a review of several studies spanning a 20-year period in cats (Wells et al., 2008), the mean duration of analgesia for all dosages and routes was 160.3 minutes with a standard deviation of ± 130.8 min. The mean dosage administered was 0.4 mg/kg. The effective plasma concentration was above 45 ng/ml from the analysis of these studies, which is maintained for approximately 3 hours at a dose of 0.4 mg/kg.

Butorphanol is effective as an antitussive when administered per os to dogs (Gingerich et al., 1983) and cats, but due to its poor bioavailability, it is not an effective analgesic unless high doses are administered (1–2 mg/kg PO q 2–4 h). The sedative effects of butorphanol are prominent and appear to occur at lower dosages than its analgesic effects. Therefore sedation following butorphanol administration should not be interpreted as analgesia (Sawyer et al., 1991). Butorphanol was previously unscheduled because of lower abuse potential compared to full μ-receptor agonists, but it is now a DEA Schedule IV drug (Table 13.2).

Butorphanol, like other opioids, causes minimal clinically relevant cardiovascular effects when administered at recommended dosages. Adverse effects are similar to other opioids including sedation, dysphoria, mydriasis (cats), decreased GI motility, and constipation. Intramuscular and subcutaneous administration is painful. The respiratory depressant effects of butorphanol appear to be less than that of opioid agonists. Interestingly, the LD_{50} of IV butorphanol in dogs, 10 mg/kg, is markedly less than pure μ-agonists including morphine HCl (175 mg/kg IV), methadone HCl (28.7 mg/kg IV), and codeine phosphate (97.8 mg/kg IV) (Kasé et al., 1959).

In horses, butorphanol has been favored over other opioids because it appears to be better tolerated. Excitatory effects, although possible, are less than with other opiates because these effects are primarily mediated via the μ-opioid receptor (see Section Central Nervous System Excitation). Decreased fecal output was observed in horses (Sellon et al., 2001, 2004), but it was transient and not as severe as with other opioids. Less severe inhibition of gastrointestinal motility in horses are probably attributed to less stimulation of the μ-opioid receptor by butorphanol (see Section Gastrointestinal Effects; DeHaven-Hudkins et al., 2008).

Doses used in horses for treatment of visceral pain are in the range of 0.1 or 0.2 mg/kg IV as a bolus. After a bolus injection, ataxia, increased locomotor activity, and decreased intestinal motility may occur. In horses, the duration of effectiveness from a single dose is short – 30 to 90 minutes – in most studies. Therefore, to maintain analgesia without producing high peak concentrations that occur with intermittent boluses, a constant dose infusion protocol has been developed by Sellon et al. (2001, 2004). In those studies the half-life in horses was short (44 minutes) and the clearance was rapid (21 ml/kg/min). To maintain a plasma concentration in the effective range (judged to be 20–30 ng/ml) a loading dose of 18 μg/kg followed by a constant rate infusion of 24 μg/kg/h was used. This treatment was well tolerated with few adverse cardiovascular effects, and less adverse effects on intestinal motility than bolus doses. Some ataxia still occurred. In a follow-up study (Sellon et al., 2004), a lower infusion dose of 13 μg/kg/h was administered to patients that had undergone abdominal surgery. These horses had decreased fecal output, but it was only transient. There was less pain in treated horses, recovery from surgery was faster, and hospitalization time was less.

Nalbuphine

Nalbuphine is a κ agonist and μ receptor antagonist with similar pharmacological effects as butorphanol. Nalbuphine is effective for mild to moderate pain and appears to be well tolerated in dogs and cats although it has not been extensively investigated. Very few studies have investigated nalbuphine in horses, but effects and adverse effects are expected to be similar to morphine and butorphanol. An advantage of nalbuphine over butorphanol is that nalbuphine is not currently a DEA scheduled drug, therefore special record keeping is not necessary. Nalbuphine exhibits similar potency and pharmacokinetics as morphine, therefore dosage recommendations are the same as for morphine. Nalbuphine can be administered to antagonize the respiratory depressant effects of opioid agonists, but maintain some degree of analgesia.

Pentazocine

Pentazocine is a κ agonist and μ receptor partial agonist with similar pharmacological effects as butorphanol and nalbuphine. Pentazocine is effective for mild to moderate pain with a 1–3 hour duration of effect. Pentazocine may cause less sedation as compared to other opioids, but similar to other opioids it can cause excitement and tremors at high doses. Pentazocine (Talwin) was once commonly used in horses, but is now rarely used. The indication approved by the FDA for horses is 0.33 mg/kg once daily IV or IM for treatment of colic pain (repeated if necessary in 10–15 minutes). The approved use in dogs was 1.67 to 3.3 mg/kg IM for treatment of postoperative pain.

Nalorphine

Nalorphine is a partial κ agonist and μ receptor antagonist which exhibits minimal analgesic effects. The efficacy of reversing opioid agonist induced respiratory depression is variable and can exacerbate it in some cases. Therefore the use of nalorphine is not recommended.

Opioid Antagonists

The primary indication for opioid antagonists is for the treatment of an opioid agonist overdose if severe respiratory depression is present or reversal of adverse effects such as severe dysphoria. The duration of antagonist effect is often shorter than that of the agonist and repeated doses are typically required. Opioid antagonists must be used extremely cautiously in animals that are painful as administration can result in acute exacerbation of pain leading to cardiovascular shock and even death. Nalbuphine and butorphanol can also be used to reverse the respiratory depressant effects of opioid agonists while maintaining some analgesia. Opioid antagonists not only reverse exogenously administered opioids, but also endogenous opioid peptides. Opioid antagonists are effective in controlling crib biting in horses, but due to the need for parenteral administration and efficacy limited to 6 hours or less, are not clinically useful. Although research suggests ultralow doses of opioid antagonists may enhance analgesic effects of μ-opioid agonist (La Vincente et al., 2008), the clinical application has not been defined and is currently not recommended until further research in animals is completed.

Naloxone

Naloxone is an opioid antagonist with greater activity at the μ receptor compared to the κ and δ receptors. Naloxone also interacts at the GABA receptor as an antagonist and as a result can elicit seizures upon administration. Naloxone is typically administered 0.01–0.04 mg/kg IV and repeated as needed every 2–3 minutes to reverse respiratory depression. Careful titration can reverse the respiratory depressant and other adverse effects of an opioid agonist while maintaining some degree of analgesia. To achieve the desired effect during titration the full dose of 0.04 mg/kg should not be administered initially. Instead, start with 0.04 mg/ml solution and administer increments of 1 ml every 30 seconds until vocalization or signs of dysphoria stop. A dose of 0.01 mg/kg IV has been given for this purpose without losing analgesic effects. A dose of 1 ml (0.4 mg) will reverse 1.5 mg oxymorphone, 15 mg morphine, 100 mg meperidine, and 0.4 mg fentanyl.

Naltrexone

Naltrexone is an opioid antagonist with activity at the μ, κ, and δ receptors. Naltrexone is most commonly used to reverse carfentanil administered at a dose of 100 mg naltrexone for 1 mg carfentanil, with one-quarter of the dose administered IV and the remaining three-quarters administered SC.

Nalmefene

Nalmefene is an opioid antagonist with greater activity at the μ receptor compared to the κ and δ receptors, similar to naloxone. However, the duration of effect is longer than that of naloxone. Nalmefene administered at 0.012 mg/kg/h was as efficacious as naloxone 0.048 mg/kg/h in antagonizing the respiratory depressant effects of fentanyl administered to dogs (Veng-Pedersen et al., 1995).

Diprenorphine

Diprenorphine (M 50/50) is an antagonist at the μ, κ, and δ receptors, similar to naltrexone. Diprenorphine is used primarily to reverse the effects of etorphine. Two milligrams of diprenorphine will reverse the effects of 1 mg etorphine.

Newer Agents

Newer antagonists are being explored for use, primarily to limit the adverse effects of opioids on gastrointestinal motility. These agents are considered *peripheral* opioid antagonists, rather than *central* opioid antagonists. Such agents include alvimopan and methylnaltrexone. They are being investigated to be used as therapeutic agents to treat gastrointestinal motility problems associated with opioid analgesic use as well as other stress or pain syndromes that cause a decrease in gastrointestinal motility (for example postoperative ileus) (DeHaven-Hudkins et al., 2008). These agents are capable of restoring gastrointestinal motility, but preserving opioid-mediated analgesia. Alvimopan has advantages over methylnaltrexone with respect to potency and duration of activity. These antagonists are discussed in more detail in Chapter 46.

Other Central Analgesic Drugs

Tramadol

Tramadol is a centrally acting analgesic eliciting it effects through a complex interaction as a μ-opiate agonist (by an active metabolite) and as serotonin (5-HT) reuptake inhibitor, noradrenergic reuptake inhibitor (α-2), and muscarinic (M1) antagonist. Tramadol is both highly lipophilic (Log P 2.5) and highly soluble (33 mg/ml), making it suitable for oral and IV administration. (There is no IV formulation commercially available in the USA at this time.)

Tramadol is administered as a racemic mixture (+ and −). It is metabolized to greater than 30 metabolites, with perhaps as many as 11 metabolites significant. The most important metabolite, with respect to pharmacological activity is the active metabolite, O-desmethyltramadol (also referred to as the M1 metabolite), which is metabolized by the CYP2D isoenzyme in many species. Concurrent administration of a CYP2D inhibitor (quinidine, fluoxetine, paroxetine, or sertraline) with tramadol significantly decreases the analgesic effects following tramadol administration. Additionally, CYP2D poor metabolizers (human) have poorly controlled pain in response to tramadol administration.

Tramadol's pharmacological effects are complex. The effects can be attributed both the (+) and (−) isomer. Tramadol (+) is a μ-opiate agonist (weak) and also inhibits serotonin (5-HT) uptake at the synapse. Tramadol (−) inhibits norepinephrine reuptake and can produce analgesic activity via α_2-receptors. The metabolite *O*-desmethyltramadol (M1) (+) is a μ-opiate agonist with potency of 200–300 times tramadol. Taken together, the effects of tramadol may accomplish *multimodal analgesia* via these mechanisms. All three mechanisms described can produce analgesia, sedation, and other pharmacological effects, but because deficiencies in metabolism to the M1 metabolite diminish analgesia in people (discussed below), the opiate-related effects of this metabolite are regarded as an important contribution to the efficacy.

Tramadol is used for mild to moderate pain in humans and its use is extrapolated to veterinary species. Sedation, nausea, and vomiting are adverse effects that may be attributed to tramadol administration. Tramadol has less effect on gastrointestinal motility compared to opioid drugs. High doses of tramadol may lower the seizure threshold; therefore, repeated high doses should be avoided in patients prone to seizures such as epileptics. Intravenous administration of *O*-desmethyltramadol to healthy dogs resulted in sedation whereas IV tramadol did not (KuKanich and Papich, 2004b). Tramadol may produce inhibition of the reuptake of serotonin in veterinary species (although this has not been confirmed). Nevertheless, concurrent administration of tramadol and other drugs that act through serotonergic mechanisms (tricyclic antidepressant, selective serotonin reuptake inhibitor, serotonin–norepinephrine reuptake inhibitor or meperidine) should be avoided because of a potential for serotonin syndrome (Mohammad-Zadeh et al., 2008).

In dogs, tramadol is rapidly metabolized following oral and IV administration, primarily to the inactive metabolite *N*-desmethyltramadol with only low concentrations of *O*-desmethyltramadol measured in the plasma (Matthiesen et al., 1998; Giorgi et al., 2009; KuKanich and Papich, 2011). The half-life of tramadol following IV and oral administration is 0.8 and 1.7 hours, respectively (Table 13.15). The half-life of *O*-desmethyltramadol is 1–2 hours following oral tramadol administration. The oral bioavailability of tramadol in fasted dogs was initial reported to be 65 ± 38%, but a recent study reported it as low as 4 ± 3% (Black et al., 2013). A population pharmacokinetic study in postoperative dogs demonstrated highly variable plasma tramadol concentration with a predicted C_{MAX} range of 47–613 ng/ml after the second dose and very low concentrations of the active metabolite M1 (Benitez et al., 2015a). Based on these data, single doses or short term administration results in unpredictable drug exposure in dogs. Decreased bioavailability occurs with repeated administration to dogs with the

Table 13.15 Tramadol pharmacokinetics in animals compared to people. References: KuKanich and Papich, 2004b; Papich and Bledsoe, 2007; Pypendop et al., 2009; Black et al., 2013; Grond and Sablotzki, 2004.

	Dog	Cat	People
Tramadol			
Half-life (hours)	0.8	2.5, 2.2	5–6
Peak concentration oral	1402 (11 mg/kg) 32 ng/ml (5 mg/kg)	536 (4 mg/kg); 914 ng/ml (5.2 mg/kg)	200–400 ng/ml (1.4 mg/kg)
Volume of distribution	3 L/kg	3 L/kg	2.8, 3.4 L/kg
Clearance (ml/kg/min)	55	21	6–7
Systemic absorption from oral dose	4–65%	93%	70%
O-Desmethyltramadol (M1)[a]			
Half-life (hours)	1.7	4.5, 4.3	6–7
Peak concentration (ng/ml) oral	<7	519 (4 mg/kg); 655 (5.2 mg/kg)	55–200 (1.4 mg/kg)
Ratio M1/ tramadol	0.003	1–1.2	0.35

[a] All of the O-desmethyltramadol (M1) pharmacokinetic parameters were derived from administration of tramadol as the parent drug.

relative bioavailability decreasing at day 7 to approximately 18% compared to the first dose (Matthiesen et al., 1998) suggestive that tramadol dosages would have to be substantially increased over time to maintain targeted drug exposure. Decreased bioavailability with multiple doses has not been reported in other veterinary species.

Human poor metabolizers of tramadol to *O*-desmethyltramadol (similar metabolism profile to dogs) had 60–80% failure rates to tramadol for postoperative pain compared to 20–30% failure rates in humans who had adequate conversion of tramadol to *O*-desmethyltramadol (Stamer et al., 2007). Consistent efficacy of tramadol in dogs has not been adequately demonstrated. Oral tramadol, 10 mg/kg, resulted in increases in mechanical thresholds in laboratory Greyhound dogs (KuKanich and Papich, 2011). However, IV tramadol at doses up to 10 mg/kg failed to affect thermal thresholds in laboratory Beagle dogs (Kögel et al., 2014). A placebo-controlled clinical trial in dogs with osteoarthritis resulted in tramadol treated dogs resulted in improved mobility by owner questionnaire, but because of high variability, failed to achieve an adequate statistical power (Malek et al., 2012). Plasma tramadol concentrations were not detected in four of the dogs after 2 weeks of therapy after 4 mg/kg PO q 8 h, and the mean concentrations were very low, indicative of induced metabolism. The low to undetectable concentrations of the active drug in these studies did not support the use of tramadol for osteoarthritis in dogs at 4 mg/kg PO q 8 h. The current dose recommendation for tramadol use in dogs is 3–5 mg/kg PO q 6–8 h, but doses up to 10 mg/kg have been administered, especially when tramadol metabolism is increased because of repeated exposure. Overall, despite the interest among clinicians for its use, there are very few data from well-designed controlled studies (either experimental or clinical) to support the administration of tramadol in dogs to treat painful conditions. Some studies that demonstrated efficacy, used current administration of a NSAID, making it difficult to evaluate the true effectiveness of tramadol (Flôr et al., 2013; Davila et al., 2013).

The efficacy of tramadol as an analgesic in cats has been reported in experimental models in which dose-dependent increases in analgesia occurred following oral tramadol 0.5–4 mg/kg (Pypendop et al., 2009). Tramadol administered to postoperative ovariohysterectomy cats required less rescue analgesia than placebo-treated cats, but cats treated with the combination of tramadol (2 mg/kg SC q 8 h) and the NSAID vedaprofen did not require any rescue analgesia (Brondani et al., 2009).

There are important differences between cats and dogs (Table 13.15). In cats there is a slower clearance and a longer half-life compared to dogs. Most importantly, the clearance of the *O*-desmethyltramadol metabolite (M1), which is conjugated to glucuronic acid for elimination in other animals, is much slower in cats. Because the clearance of M1 is slower in cats than in dogs, the ratio of M1 to tramadol is 1 : 1 or higher in cats (Table 13.15). The M1 metabolite is associated with greater opiate-mediated effects than the parent drug, therefore effects attributed to opiates are observed more often in cats than dogs. Because of the increased effects in cats, the doses should be lower than in dogs.

In anesthetized cats, tramadol at a dose of 4 mg/kg IV produced a dose-dependent respiratory depression, which was partially reversed by naloxone (Teppema, 2003). Tramadol has also demonstrated efficacy as an antitussive agent in cats, which may be attributed to the M1 metabolite (Nosal'ova et al., 1991; Pypendop and Ilkiw, 2008). Doses of 4–5 mg/kg orally produce some euphoria to dysphoria and mydriasis in experimental cats (Papich and Bledsoe, 2007; Pypendop and Ilkiw, 2008). Dose recommendations for cats have been in a range of 2–4 mg/kg PO q 8–12 h, but further work is necessary to determine the optimum dose and frequency. Oral tramadol is notoriously unpalatable in cats and causes salivation and resistance to medicate; therefore, repeated administration can be challenging.

The pharmacokinetics and metabolism of tramadol in horses is quite variable. Tramadol IV is rapidly eliminated (half-life 1–2 hours) with low concentrations of M1 measured (Stewart et al., 2011; Giorgi et al., 2007). The oral bioavailability of the immediate release formulations were relatively high in one study 60–70% (Girogi et al., 2007), but low (~10%) in another (Stewart et al., 2011). The plasma concentrations of M1 are also quite variable between studies after PO tramadol and may be due to differences in conjugation of M1 (Guedes et al., 2014). Multiple doses of oral tramadol at 10 mg/kg q 12 h produced plasma tramadol concentrations within a potential therapeutic range in horse, but M1 concentrations were relatively low (Guedes et al., 2014). Tramadol was well tolerated at 10 mg/kg PO q 12 h for 5 days based on behavior, blood pressure measurements, heart rate respiratory rate, and gut sounds. However, the horses objected to oral tramadol administration, which may be due to the bitter taste of tramadol. One horse developed colic on day 4 of the study, which resolved after skipping one dose and with periodic walking and it is not clear if that was directly due to tramadol or the stresses of the study. The horse was administered the remaining doses of tramadol and finished the study without further adverse effects. Horses with chronic laminitis administered oral tramadol 10 mg/kg q 12 h for 7 days had significant improvement in forelimb off-loading and achieved reasonable tramadol and M1 maximum plasma concentrations with no adverse effects reported (Guedes et al., 2016). Therefore, oral tramadol may have some use as an analgesic in horses, but the efficacy may be dependent on an individual animal's plasma M1 concentrations, which are variable.

Tramadol (Ultram, and generic) is available in 50-mg tablets. An extended release tablet (Ultram-ER) also has been examined in dogs (Papich et al., 2007). A single 300-mg tablet produces sustained plasma concentrations in dogs in excess of 24 hours but the concentrations of the extended release tablet were much lower than comparable doses used in people. There are no commercially available injectable formulations in the USA, but are available in some other countries.

Other Routes of Administration

In order to maximize analgesic effects and minimize adverse effects, other routes of opioid administration have been evaluated. The two routes most commonly utilized are epidural and synovial administration.

Epidural

Opioids administered by the epidural route interact with spinal opiate receptors to elicit an analgesic response. Epidural opioid administration does result in systemic absorption of the opioid, but at lower concentrations than parenteral administration because the total dose (mg/kg) is lower. However, the high spinal concentrations achieve profound spinal analgesia. Because of their lipophilicity, some opioids can diffuse from the epidural space to the plasma. The high lipophilicity of sufentanil and alfentanil (and presumably fentanyl) also result in a more rapid effect (and shorter duration) than epidural morphine (Tiseo et al., 1990). Morphine is the most common opioid administered by the epidural route and has produced a duration of effect up to 24 hours. Epidural administration results in decreased systemic adverse effects such as sedation and dysphoria, but other adverse effects such as pruritus, urine retention, and constipation can still occur. The efficacy is probably due to the very high concentrations of morphine interacting with the spinal receptors. Preservative-free morphine solutions are available for epidural administration as the preservatives (such as phenol and formaldehyde) can cause toxicity and adverse effects if administered for multiple doses. However, some experts state a single dose of preservative-containing solutions have been administered to dogs. Techniques for administering opioids via this route in veterinary species are provided in more detail in other chapters (Skarda and Tranquilli, 2007).

Peripheral

Opiate receptors have been identified in the synovial membranes of various species including the horse and dog (Sheehy et al., 2001; Keates et al., 1999). Intaarticular administration of morphine following endoscopic procedures produces a significant analgesic effect as compared to a placebo in humans (Lawrence et al., 1992). Low plasma concentrations are attained following intraarticular administration presumably resulting in fewer adverse effects. Dose titration studies or placebo controlled studies of intraarticular morphine in horses have not been reported. Intraarticular morphine 0.1 mg/kg diluted to a volume 0.5 ml/kg appears to decrease the need for supplemental analgesia following stifle surgery in dogs (Sammarco et al., 1996). Other techniques for local administration of opioids are provided in more detail in other texts (Skarda and Tranquilli, 2007).

The Use of Opioids in Nonmammalian Vertebrates

The pharmacology of opioids in nonmammal species is not as abundant as for the domestic mammal species, but opioid receptors are apparently highly conserved among groups of vertebrates (mammals, birds,

Table 13.16 Relative supraspinal opiate receptor density in different species

	μ	κ	δ
Frog brain	37%	19%	44%
Chicken forebrain	58%	25%	17%
Pigeon brain	14%	76%	10%
Junco (passerine) brain	74%	22%	4%
Rat forebrain	41%	9%	50%
Mouse forebrain	25%	13%	62%
Guinea Pig forebrain	25%	50%	25%
Dog cortex	5%	58%	37%
Human cortex	29%	37%	34%

frogs, and fish) (Li et al., 1996). μ, κ, and δ opiate receptors have been identified in birds' brains as high affinity, but appear to be low in density as compared to mammals. The relative density of opioid receptors also appears to be dependent on the species of bird evaluated (Table 13.16). Therefore a drug and dosage effective in parrots may or may not be effective in doves, finches, waterfowl, ratites, etc. The limited studies available have demonstrated a wide range of doses and effects. Some studies have evaluated the efficacy of opioids in companion birds. Butorphanol (1 mg/kg IM) decreased the MAC of isoflurane in cockatoos, which may be indicative of analgesic effects (Curro et al., 1994). The duration of effect or different doses were not evaluated. In a separate study, butorphanol (1 mg/kg IM) resulted in a greater effect than buprenorphine (0.1 mg/kg IM) in African Grey Parrots (Paul-Murphy et al., 1999). However, only a single dose for each drug was assessed, the birds were evaluated to only 60 minutes, and neither drug was effective in greater than 50% of the animals assessed at 60 minutes. Butorphanol 2 mg/kg IM has no significant effects on electrical nociceptive stimulus in Hispaniolan Amazon parrots (Sladky et al., 2006). Nalbuphine (12.5, 25, 50 mg/kg IM) administered to Hispaniolan Amazon parrots produced significant effects for 1.5–3 hours after administration using a thermal model (Sanchez-Migallon Guzman et al., 2011). Buprenorphine (0.1, 0.3, 0.6 mg/kg IM) resulted in significant antinociceptive effects for at least 6 hours using a thermal model, which were approximately dose proportional in American kestrels (Ceulemans et al., 2014). Butorphanol (1, 3, 6 mg/kg IM) failed to produce significant antinociceptive effects in American kestrels using a thermal model (Guzman et al., 2014a). Tramadol 30 mg/kg PO produced thermal antinociception in Hispaniolan Amazon parrots for up to 6 hours (Sanchez-Migallon Guzman et al., 2012). Tramadol (5, 15, 30 mg/kg PO) resulted in significant antinociceptive effects in American kestrels for up to 9 hours at 5 mg/kg, but the effect was less pronounced at the higher doses (Guzman et al., 2014a).

Table 13.17 The dose required to elicit a 50% maximum analgesic effect (ED_{50}) and lethal dose (LD) of opioids in tree frogs

	ED_{50} (mg/kg)	LD (mg/kg)
Morphine	24.6	ND
Fentanyl	0.47	30
Methadone	6.1	1000
Meperidine	31.7	ND
Buprenorphine	46.4	ND

ND, not determined.

The effects of various opioids have been studied in amphibians (grass frogs). A single study evaluated the antinociceptive effects of morphine, methadone, meperidine, fentanyl, and buprenorphine in an experimental model (Table 13.17). The opioids were administered SC into the dorsal lymph sac. The effective dose to elicit a 50% maximum analgesic response (ED_{50}) was determined as well as the lethal dose (LD) of some of the compounds (Stevens et al., 1994). The analgesic effects lasted for at least 4 hours, which was the last time point evaluated. In turtles, butorphanol did not provide an antinociceptive effect, even when administered at a high dose of 2.8 and 28 mg/kg, but it produced respiratory depression (Sladky et al., 2007). However, in the same turtles, morphine at 1.5 or 6.5 mg/kg produced significant antinociceptive effects. Morphine at these doses caused long-lasting respiratory depression. At these doses the respiratory depression may limit clinical use. Hydromorphone (0.5 mg/kg SC) elicited antinociceptive effects with effects lasting at least 24 hours, but less than 48 hours (Mans et al., 2012). However, in the same study, buprenorphine (0.2 mg/kg SC) did not elicit antinociceptive effects in red-eared slider turtles using a thermal stimulus model. These studies in turtles indicate that the population of opioid receptors may be different in turtles compared to other vertebrates with μ-opioid receptors providing more prominent effects than κ or δ(Sladky, 2009). Tramadol provided significant antinociceptive effects in red-eared sliders using a thermal model at least up to 24 and 96 hours for 5 and 10 mg/kg, respectively (Baker et al., 2011). In contrast to morphine, respiratory depression effects were not observed with tramadol.

Morphine 10 and 20 mg/kg SC resulted in significant antinociception for up to 8 hours in Bearded dragons using a thermal stimulus, but butorphanol did not produce significant effects up to 20 mg/kg IM (Sladky et al., 2008). In contrast, butorphanol 20 mg/kg IM in corn snakes resulted in significant antinociceptive effects to at least 8 hours, but morphine up to 40 mg/kg IM did not result in effects using a thermal model (Sladky et al., 2008).

The analgesic effects of morphine and meperidine were evaluated in crocodiles (Kanui and Hole, 1992).

Morphine, 0.3 mg/kg IP, was the dose that produced the maximum analgesic effect in the experimental model. Morphine, 0.1 mg/kg IP, produced significant analgesic effects from 30 minutes to 2 hours. The analgesic effect following the higher dose, 0.3 mg/kg, would be expected to last longer, but the duration of the higher dose was not assessed. Meperidine produced a maximum response at a 2–4 mg/kg IP dose, and 2 mg/kg IP resulted in significant analgesia from 30 minutes to 3 hours.

The effects of butorphanol, 1 mg/kg IM, on the minimum alveolar concentration of isoflurane were evaluated in green iguanas (Mosley et al., 2003). No significant reduction in the MAC occurred, but only a single dose was assessed. A separate study demonstrated butorphanol, 1 mg/kg, had no significant cardiovascular effects in iguanas when administered to isoflurane-anesthetized animals (Mosley et al., 2004).

The effects of opioids have not been extensively evaluated in fish due to inherent difficulties evaluating pain in fish. Morphine 0.3 mg/g (300 mg/kg) appeared to produce an analgesic response in an experimental rainbow trout model, but the duration of the effect was not evaluated (Sneddon et al., 2003). The metabolism and analgesic effects of morphine in goldfish were evaluated following a bath administration (Jansen and Green, 1970). No metabolites were identified and recovery of unchanged morphine accounted for nearly 100% of the administered dose. A morphine bath consisting of 0.56 mg of morphine per 1000 ml water resulted in maximal inhibition of an electrical stimulus model in 5/5 fish, however tolerance was rapidly noted with repeated exposure every 3 days. Jansen and Green reported apparent CNS excitement with higher doses similar to mammals. In winter flounder, morphine at high doses of 40 mg/kg IP or 17 mg/kg IV produced marked cardiovascular depression that lasted for 48 hours (Newby et al., 2007). Morphine produced antinociceptive effects in rainbow trout using an electrical stimulus, which was blocked by naloxone (Jones et al., 2012). A dose–response relationship was document with a reported ED_{50} of 6.7 mg/kg IP and effects plateaued at approximately 30 mg/kg.

Harms et al. examined the effects of butorphanol and ketoprofen in postoperative Koi. Butorphanol, 0.4 mg/kg IM, produced a significant difference in postoperative behavior in Koi carp as compared to ketoprofen and saline, which may be indicative of an analgesic response (Harms et al., 2005). Butorphanol (10 mg/kg IM) and morphine (5 mg/kg IM) appeared to produce analgesia in postoperative Koi undergoing gonadectomy and morphine was associated with fewer deleterious effects (Baker et al., 2013).

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14

Sedatives and Tranquilizers

Lysa P. Posner

Introduction

Sedatives and tranquilizers are commonly used in veterinary medicine to produce calmness, for chemical restraint, and as an adjunct with general anesthesia. Drugs that produce sedation often have different mechanisms of actions, pharmacodynamic effects, analgesic properties, reversibility, and physiological effects. It is therefore important to understand the different classes of sedative available to be able to predict wanted effects and potential side effects.

Phenothiazine Derivatives

Introduction

Phenothiazines were originally developed and used as antipsychotic drugs for the treatment of schizophrenia in people. However, they have been used in veterinary medicine as tranquilizers since the 1950s. The term *neuroleptic* (antipsychotic) was first used to describe the effects of phenothiazines on the central nervous system, but the more common term, *major tranquilizer*, is still used. The most commonly used phenothiazine in veterinary medicine is acepromazine (ACP). It is incorrectly referred to as "acetylpromazine" in some publications. It is unique because it is not used in human medicine, but has been widely used in veterinary medicine for decades.

Chemistry

Classification: Phenothiazine derivative, major tranquilizer, and antiemetic.

Mechanism of Action

Phenothiazine drugs produce sedation by inhibiting postsynaptic central dopaminergic receptors (D2). Dopamine receptors are included in the family of G-protein-coupled receptors and act as a first messenger by interacting with the receptor proteins of the postsynaptic membrane. This interaction results in transduction of the signal by a guanine nucleotide-binding regulatory protein (G protein) to an appropriate intracellular effector system, or second messenger. Therefore antagonism of the D_2 receptor decreases neurotransmission resulting on behavioral changes (calming and sedation).

Phenothiazines also have antiemetic effects, which are from D2 antagonism at the chemoreceptor trigger zone (Peroutka and Snyder, 1982). Peripherally, and as a side effect, the phenothiazines block the binding of norepinephrine at α-adrenergic receptors (α_1 antagonism). Blockade of these receptors results in peripheral vasodilation.

Indications

Phenothiazines are commonly administered for routine sedation in a variety of species. They are also used in the perianesthetic period to reduce anxiety, reduce induction and maintenance drugs doses, decrease vomiting in susceptible animals, improve the quality of induction and recovery, and to contribute to more balanced anesthesia. Phenothiazines are commonly administered with opioids (e.g., morphine) to produce *neurolept analgesia*; the combination produces synergic effects of each class (i.e., greater sedation and greater analgesia). Phenothiazines can also prevent nausea and vomiting, particularly the vomiting associated with opioid administration (Valverde et al., 2004).

Physiological Effects

Central nervous system effects: All phenothiazines produce a sedation by depressing dopaminergic neurotransmission but vary in potency and duration of action. Phenothiazines decrease spontaneous motor activity in animals, but should not affect coordination. Although sedation is generally reliable, arousal is easily accomplished, particularly in excited or aggressive animals. Extrapyramidal symptoms (rigidity, tremor, akinesia)

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can be an adverse effect of phenothiazines, particularly at high doses.

Phenothiazines as a class (including acepromazine) have been considered contraindicated for patients with seizure histories, due to a series of experiments in rabbits that showed alteration of the seizure threshold with a proconvulsant drug in patients administered chlorpromazine (Fabisch, 1957). However, there is little scientific evidence that acepromazine induces spontaneous seizures in veterinary patients and recent studies have disputed this conclusion and have even suggested that acepromazine may be anticonvulsant (Tobias et al., 2006; McConnell et al., 2007).

Cardiovascular effects: In addition to blockade of the central effect of dopamine, phenothiazines block peripheral α_1-adrenergic receptors in the vasculature, which results in peripheral vasodilation, and can produces systemic hypotension and a decrease in cardiac output (Stepien et al., 1995). Healthy animals show mild changes to arterial blood pressure; however, the hypotensive effects may be exaggerated in patients that are anesthetized, debilitated, or hypovolemic. A mild reflex tachycardia may be seen in response to hypotension (Turner et al., 1974). Vasoconstrictors, such as phenylephrine, can be used to attenuate the hypotensive effects of phenothiazines (Ludders et al., 1983). The vasodilatory effects generally last longer than the sedation.

Phenothiazines should be used cautiously with locoregional (epidural and intrathecal) anesthetic procedures because they might potentiate the arterial hypotensive effects of local anesthetics. Acepromazine decreases ventricular arrhythmias by raising the arrhythmogenic dose of epinephrine (Muir et al., 1975), even at low doses (Dyson and Pettifer, 1997).

Respiratory effects: Clinical doses of phenothiazines ordinarily have mild effects upon respiratory activity. Respiratory rate is often depressed, but minute volume remains normal (i.e., no change in CO_2). However, respiratory depression may be seen or exaggerated when phenothiazines are administered with other CNS or respiratory depressants (e.g., opioids, isoflurane) or when used at high doses.

Acepromazine attenuates the shunt of ventilation perfusion mismatches seen horses sedated or anesthetized with α_2-agonists and or dissociative anesthetics (Marntell et al., 2005) (see Section Species Differences, Horse).

Musculoskeletal effects: Phenothiazines provide good muscle relaxation and are often used in conjunction with anesthetics that do not provide muscle relaxation or that result in muscle rigidity (e.g., ketamine). The phenothiazine, acepromazine, has been shown to decrease the incidence of malignant hyperthermia in pigs (see Section Species Differences).

Pharmacokinetics and Metabolism

Phenothiazines in general have a large volume of distribution (Vd) and are highly protein bound. In the horse, acepromazine Vd = 6.6 l/kg and is >99% protein bound (Ballard et al., 1982). Time to clinical effect is generally ~10–15 minutes after IV administration and ~30 minutes after IM administration. Acepromazine is long acting in most species; elimination half-life is 3.1 hours in the horse and 7.1 hours in the dog (Ballard et al., 1982; Hashem et al., 1992). Acepromazine undergoes extensive hepatic metabolism and metabolites are excreted in the urine (Dewey et al., 1981). The metabolites of acepromazine have not been evaluated extensively in most species, but horses and humans have differing metabolites and in differing concentrations (Dewey et al., 1981; Elliott and Hale, 1999). Bioavailability in dogs after oral administration is ~20% (Hashem et al., 1992), which must be accounted for if tablets are administered instead of injection. There is no available antagonist to reverse the effects of phenothiazines.

Miscellaneous

Equine recovery from anesthesia: Acepromazine use is associated with decreased perianesthetic morbidity and mortality in horses (Johnston et al., 2002).

Thermoregulation: Patients administered phenothiazines cannot thermoregulate appropriately. Phenothiazines alter thermoregulation by decreased catecholamine binding in the hypothalamus (where thermoregulation is controlled centrally) as well as by altering vasomotor tone in the peripheral vessels that participate in heat retention and elimination. Following administration, patients should be protected from extreme temperature fluctuations (e.g., housed in the sun or in the cold).

Platelet aggregation: The use of acepromazine in patients with coagulopathies is controversial. Acepromazine has been shown to decrease platelet aggregation, which alters coagulation times (Barr et al., 1992). Conversely, administration of acepromazine did not change platelet function via thromboelastography (Conner et al., 2012). Clinically, acepromazine has rarely been implicated in abnormal bleeding. Recommendations for acepromazine use are clinician dependant, although careful consideration in patients with abnormal number or function of platelet, or overt coagulopathy would be prudent.

Hematocrit: Phenothiazines markedly reduce the hematocrit of animals (Parry and Anderson, 1983; Robertson et al., 2001; Ballard et al., 1982). The decrease in packed-cell volume (PCV) by phenothiazines is likely due to splenic sequestration of red blood cells as well as translocation of fluids in response to hypotension. Blood samples drawn from phenothiazine-treated animals for diagnostic purposes should be interpreted accordingly.

Antiemetic: Phenothiazines are antiemetic and have been shown to decrease vomiting in patients when administered with drugs that cause vomiting (e.g., opioids) (Valverde et al., 2004). The antiemetic effects are presumably from the blockade of dopamine receptors in the chemoreceptor trigger zone within the medulla (Peroutka and Snyder, 1982).

Phototoxicity: Phenothiazine derivatives can produce phototoxicity (Elisei et al., 2002), which has been induced in laboratory animals following administration of chlorpromazine in the presence of black-light irradiation (Akin et al., 1979). Although phototoxic reactions in animals do not appear to be an important clinical problem, those with scant hair and white hair probably should not receive excessive exposure to sunlight during treatment with phenothiazines.

Antihistamine: Phenothiazine drugs have antihistaminic (H1) properties (Lichtenstein and Gillespie, 1975). These effects are clinically relevant and these drugs should not be used for skin testing where histamine-caused wheal formation is assessed (Moriello and Eicker, 1991). Although potentially useful for preventing histamine release, the hypotension associated with phenothiazine drugs would preclude their use to treat abnormal histamine release (e.g., mast cell degranulation).

Analgesia: Phenothiazine derivatives provide little or no analgesic activity (Barnhart et al., 2000). Tranquilization with these drugs must be supplemented with analgesics and/or general anesthetics to block nociceptive responses during painful procedures.

Specific Drugs

Many phenothiazines are used to treat psychiatric problems in people and animals. The following three phenothiazines are currently used in veterinary medicine to produce sedation or as an adjunct to anesthesia.

Acepromazine Maleate

Acepromazine is the most commonly used phenothiazine in veterinary medicine. It is routinely used for sedation of many veterinary species, although it only approved for use in dogs, cats, and horses. It is approved for administration IV, IM, or SQ.

Synonym: Acetylpromazine, Vetranquil, ACE, ACP.

Systematic chemical name: 1-[10-[3-(dimethylamino)propyl]-10H-phenothiazin-2-yl ethanone.

Molecular formula: C19-H22-N2-O-S (Figure 14.1).

Molecular weight: 442.5334.

How supplied: Acepromazine is available in yellow aqueous solution at 10 mg/ml and in 5, 10, and 25-mg tablets. The solution should be protected from light.

Dosage for acepromazine: see Box 14.1.

Box 14.1 Dose for acepromazine

Species	Dose and route
Dog and cat	Parenteral sedation: 0.01–0.05 mg/kg IV, IM or SQ Oral sedation: 0.5–2.0 mg/kg PO q 6–8 hours
Horse	Parenteral sedation: 0.01–0.05 mg/kg IV, IM or SQ
Pigs	Parenteral sedation: 0.03–0.2 mg/kg IV, IM, SQ
Cattle	Parenteral sedation: 0.01–0.1 mg/kg IV, IM, SQ
Sheep and goats	Parenteral sedation: 0.05–0.1 mg/kg IV, IM, SQ
Rabbits	Parenteral sedation: 1.0 mg/kg IM, SQ

Promazine Hydrochloride

Promazine is phenothiazine similar to acepromazine. It is rarely used in the parenteral form, but is more popular as a granular oral medication that is mixed with feed for use in horses.

Synonym: Promazine granules, promazine.

Name: 10-(3-dimethylaminopropyl)phenothiazine (Figure 14.1)

Molecular formula: C17-H20-N2-S.Cl-H.

Molecular weight: 442.5334.

How supplied: Promazine granules, 27.5 mg/gram for mixing with feed

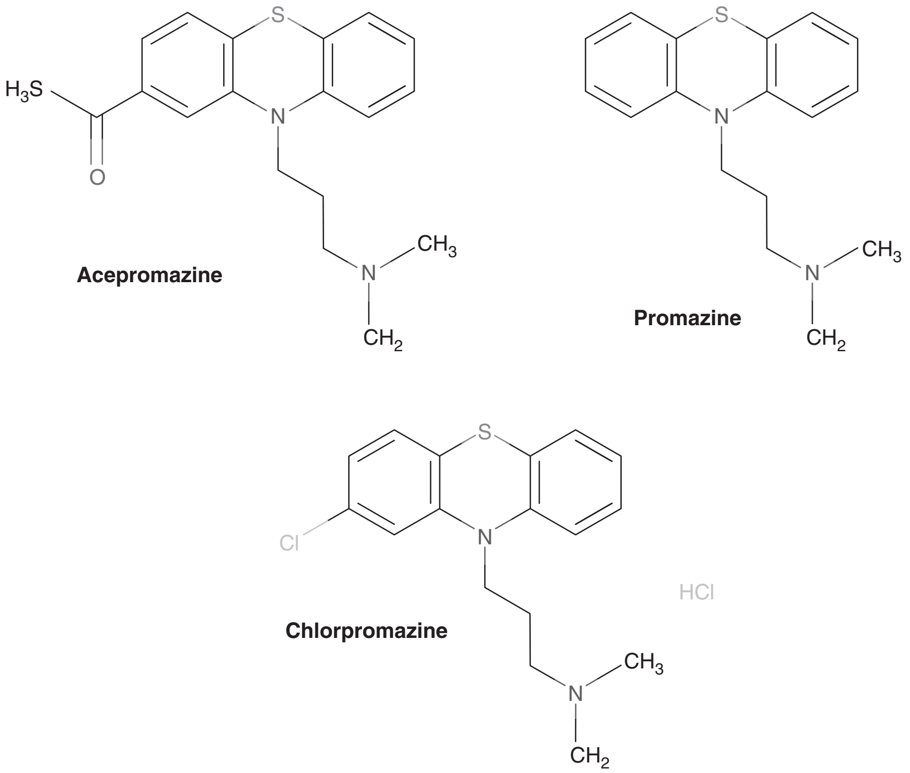


Figure 14.1 Structure of phenothiazines.

Dosage for promazine granules: For horses, mix 1–2 mg/kg of granules with feed. For cattle, mix 1.5–2.75 mg/kg with feed.

Chlorpromazine Hydrochloride

Chlorpromazine is primarily used as an antiemetic in dogs and cats, and less commonly used as a preanesthetic sedative. It is generally contraindicated for use with horses, due to a high incidence of ataxia and altered mentation.

Chemical name: 10-(3-Dimethylaminopropyl)-2-chlorophenothiazine monohydrochloride.

Molecular formula: C17-H19-Cl-N2-S.Cl-H (Figure 14.1).

For the dog and cat, administer 0.55–2.0 mg/kg IV, IM to produce sedation.

Species Differences

Horse: The use of acepromazine in the perianesthetic period is associated with decreased risk of anesthetic morbidity and mortality in horses (Johnston et al., 2002). Acepromazine have been associated with prolonged paraphimosis (penile prolapse) in the horse. Routine sedative doses are generally associated with paraphimosis, which generally resolves within 30 minutes (Ballard et al., 1982). However, in some horses prolapse can remain for greater than 100 minutes, causing swelling (likely in association with vasodilation), trauma, and failure for the penis to retract normally, which creates a management issue and potential for loss of breeding function. In the USA, acepromazine is used with caution in breeding horses, but that concern may be overrated (Driessen et al., 2011). In many parts of the world (e.g., United Kingdom), acepromazine is routinely used for equine anesthesia and has even been associated with better anesthetic outcomes in horses (Johnston et al., 2002). Anecdotal reports suggest pathological paraphimosis does not occur when the total dose does not exceed 10 mg/horse, which was supported retrospectively (Driessen et al., 2011).

Acepromazine attenuates the shunt of ventilation–perfusion mismatches seen horses sedated or anesthetized with α_2-agonists and/or dissociative anesthetics (Marntell et al., 2005). The pulmonary effects are likely due to the combination of improving cardiac output and permitting normal hypoxic pulmonary vasoconstriction (Marntell et al., 2005). Acepromazine produces α_1-adrenergic blockade, which balances the vasoconstriction of α_2-agonist sedative drugs. Vasoconstriction and the reflex bradycardia associated with α_2-agonists are major causes of decreased cardiac output. Additionally, the rise in pulmonary arterial pressure (PAP) associated with α_2-agonists and dissociative anesthetics likely interfere with hypoxic pulmonary vasoconstriction. Hypoxic pulmonary vasoconstriction is the normal mechanism to limit ventilation and perfusion mismatches.

Chlorpromazine is not recommended in horses due to extreme ataxia and altered mentation.

Dog: Some Boxer dogs have an exaggerated response to the phenothiazine, acepromazine (Brock, 1994). In those dogs, sedation and hypotension are greater than expected and there have been anecdotal reports of syncopal episodes, presumably from hypotension. Aggressive symptomatic treatment of hypotension has resulted in full recoveries. Therefore acepromazine might be avoided all together in Boxer dogs or used at much reduced doses with monitoring for any untoward effects.

Dogs with high vagal tone (Bulldogs, Boxers) have anecdotally been reported to have greater morbidity and mortality when administered acepromazine, presumably caused by the adrenergic blocking effects of the drug. Similar recommendations for avoidance or reduced dosages have been made.

Pig: Acepromazine may prevent the occurrence of halothane-induced malignant hyperthermia in susceptible pigs (McGrath et al., 1981).

Drug Interactions

All phenothiazines should be used cautiously with any drugs that also produce vasodilation or hypotension. All phenothiazines when administered with other CNS depressants can produce exaggerated CNS depression. Doses of one or both drugs might need to be reduced.

Overdose/Acute Toxicity

The phenothiazines have very high therapeutic indexes, particularly when used in conscious (nonanesthetized) animals. (Stoelting, 1999). Dogs that received more than 100 times the recommended oral dose had no fatalities. This safety margin is likely diminished in anesthetized patients where many protective reflexes are attenuated.

Contraindications

Phenothiazines should be avoided in patients that are dehydrated, hypovolemic, bleeding, or in shock because of the drugs effect on vessel tone (vasodilation). Phenothiazines should be used cautiously in patients with coagulopathies or thrombocytopenia due to their effect on platelet aggregation (see Section Miscellaneous). Phenothiazines should be used cautiously in Boxer dogs, brachiocephalic dogs, breeding stallions, or debilitated animals.

Regulatory Information

Controlled drug status: Acepromazine is a nonscheduled drug.

Withdrawal times: No listed withdrawal times for the dog, cat, or horse; however, phenothiazines should not to be used in horses intended for human consumption.

Veterinary Approved Products (Representative)

Acepromazine

Acepromazine is approved by the FDA for use in dogs, cats and horses.

Acepromazine Maleate Injection 10 mg/ml (Boehringer Ingelheim Vetmedica, Inc.).
Acepromazine Maleate 10 or 25-mg Tablets (Boehringer Ingelheim Vetmedica, Inc.).

Promazine

Promazine is approved by the FDA for use in dogs, cats, and horses.

Promazine HCl Injectable 50 mg/ml (Zoetis)
Promazine HCl Granules 10.25 oz containers, 27.5 mg/gram (Zoetis).

Chlorpromazine

There are no veterinary approved products. It is supplied as 25 mg/ml injection for human use (Thorazine, and generic brands), and is used off label in veterinary patients. It is also supplied in 10, 25, 50, 100, and 200-mg tablets for people.

Butyrophenone Derivatives

Introduction

Butyrophenone drugs (haloperidol and droperidol) were first introduced into human medicine in the late 1950s as antipsychotics. Haloperidol is still used as an antipsychotic and droperidol is primarily used to prevent postoperative nausea and vomiting. Droperidol was used in veterinary medicine primarily as a sedative, but is no longer marketed due to adverse behavioral effects. There was previously a formulation called “Innovar-Vet”, which was a combination of droperidol and fentanyl citrate for injection, but this product is no longer marketed. More information regarding droperidol may be found in previous editions of this text. Butyrophenones are less commonly used in veterinary medicine; however, azaperone is still used for sedation in veterinary medicine in swine and zoo/wildlife medicine.

Chemistry

Classification: Butyrophenones are neuroleptic sedatives, and still one of the major classes of antipsychotic drugs in use in human medicine.

Mechanism of Action

The CNS effects of butyrophenones are primarily due to the antagonism of D_2 receptors in mesolimbic–mesocortical pathways in the brain, but there is also antagonism at D_1, 5-HT, α_1, and histamine receptors (Potter and Hollister, 2001). Some of the adverse signs associated with butyrophenones, such as extrapyramidal signs, tardive dyskinesis, muscle tremors, and restlessness, are associated with dopaminergic antagonism of the nigrostriatal pathways (Potter and Hollister, 2001). Although butyrophenones produce α_1-adrenergic receptor antagonism (vasodilation), there is less affinity for these receptors, which explains why butyrophenones do not cause the same degree of hypotension as compared to the phenothiazines (Stoelting, 1999).

Indications

Azaperone is used mainly in swine for its calming effects when mixing weanlings and feeder pigs, to prevent maternal aggression, for transportation, and for obstetrical conditions. Azaperone has also been used as an anesthetic adjunct and sedative in a number wildlife species (Morkel et al., 2010; Radcliffe et al., 2000; Williams et al., 1981; Still et al., 1996).

Azaperone

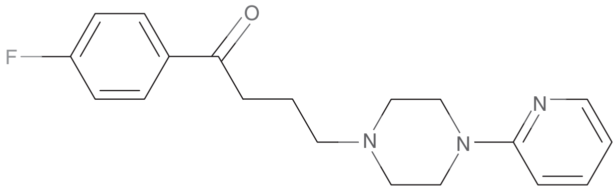
Synonym: Stresnil, Suicalm, Fluoperidol, Eucalmyl, Sedaperone.

Systematic chemical name: 1-(4-fluorophenyl)-4-(4-pyridin-2-ylpiperazin-1-yl)butan-1-one (Figure 14.2).

Molecular weight: 327.4 g/mol.

Molecular formula: C19-H22-F-N3-O.

The water solubility is 131 mg/l.



Azaperone

Figure 14.2 Structure of azaperone.

Pharmacodynamic Effects

Central nervous system effects: Azaperone produces varying levels of sedation depending on species and dose. Its effects range from behavior modification to chemical restraint (Radcliffe et al., 2000; Nishimura et al., 1993; Clutton et al., 1997; Serrano and Lees, 1976). However, there have been isolated reports of paradoxical excitement following intravenous administration of azaperone in the horse (Dodman and Waterman, 1979).

Cardiovascular effects: Azaperone causes a decrease in blood pressure, presumably from reduction in systemic vascular resistance from α_1-adrenergic blockage (Clarke, 1969). The hypotension may be associated with an increase in heart rate. It is unclear if the increase in heart rate is a response to hypotension or from a central vagolytic effect (Nashan et al., 1984; Serrano and Lees, 1976), or both. The cardiovascular changes lasted longer than the sedative effects of azaperone.

Respiratory effects: Azaperone generally produces mild respiratory depression. Respiratory depression may be enhanced when combined with other CNS depressants. Interestingly, there have been reports of increase in respiratory rates in rats, horses, and pigs (Fish et al., 2011).

Musculoskeletal effects: Azaperone produces muscle relaxation similar to the phenothiazine, acepromazine.

Miscellaneous: Packed cell volume (PCV) decreases following administration. The fall in PCV is presumably secondary to vasodilation, which would result in splenic sequestration of erythrocytes and translocation of fluid into the intravascular space in response to hypotension.

Analgesia: Butyrophenones do not interrupt the pain pathway and azaperone did not produce any analgesia in a mechanical nociceptive stimulus model in laboratory animals (Mataqueiro et al., 2004).

Pharmacokinetics Properties

There is limited information regarding the pharmacokinetics of the butyrophenones in veterinary species. The onset time after intramuscular injection is less than 10 minutes in most species with a peak effect seen approximately 30 minutes after administration (Serrano and Lees, 1976). The duration of action is 2–4 hours in pigs, longer in older pigs. Azaperone in pigs is biotransformed in the liver with 13% excreted in the feces. In rats, 25% of the drug in removed via the kidney and 10% is excreted unchanged (Fish et al., 2011). Metabolite residues are highest in the kidneys and are present in the urine for at least 3 days after administration;

however, most of the drug is eliminated from the body with 16 hours (Arneth, 1985).

Adverse Effects/ Contraindications

Thermoregulation: The ability to thermoregulate is decreased in patients administered butyrophenones. The effect on thermoregulation is a combination of α_1-receptors blockage (vasodilation) as well as antidopaminergic effects in the thermoregulatory centers in the medulla. Care should be taken when these drugs are administered to patients in cold temperatures as their normal compensatory response of vasoconstricting vessels to conserve heat loss is compromised.

Dysphoria and akathisia: Butyrophenones may produce dysphoric effects, especially in patients with a high level of anxiety. Humans describe a feeling of restlessness (akathisia), which may manifest as pacing and agitation in veterinary patients. Extrapyramidal signs are seen in 1% of human patients with the use of droperidol. These droperidol-induced extrapyramidal signs may be treated with diphenhydramine IV (Stoelting, 1999). Muscle tremors, spasticity, and irritability have been seen in the dog after the use of high doses in the range of 11–22 mg/kg IV. Salivation, sweating, muscle tremors, and vocalization has been reported in the horse following intravenous administration of azaperone at a dose of 0.29–0.57 mg/kg (Dodman and Waterman, 1979).

Paraphimosis: Doses of azaperone greater than 1 mg/kg IM may cause penile prolapse in boars, which may predispose to injury. Paraphimosis is presumably secondary to vasodilatory effects.

Representative Veterinary Dosages

See Box 14.2.

Box 14.2 Doses for azaperone

Species	Dose and route	Reference
Swine	0.4 – 2.2 mg/kg IM	
Horse	0.4 – 0.8 mg/kg IM (Intravenous administration is not recommended due to the paradoxical excitement seen in a high percentage of horses)	(Dodman and Waterman, 1979)
Deer	0.3 mg/kg IM in combination with xylazine 1 mg/kg IM	(Read and McCorkell, 2002)
Rhinoceros	0.04 mg/kg IM	(Radcliffe et al., 2000)
Elephant	50–120 mg per elephant IM	(Stegmann, 1999)

How supplied: Azaperone comes as a clear, pale yellow sterile injectable solution with a concentration of 40 mg/ml in a 100 ml multidose bottle. Azaperone should be discarded 28 days after the multidose vial has been opened.

Drug Interactions

Butyrophenones potentiates the effects of other anesthetic agents (CNS depressants), which will necessitate a reduction in the dose of induction and inhalational agents (Geel, 1991; Bustamante and Valverde, 1999; Nunes et al., 2001; Yamashita et al., 2003). Butyrophenones inhibit the effect of dopamine on renal blood flow (Bradshaw et al., 1980). The use of butyrophenones in patients receiving selegiline may precipitate extrapyramidal movement signs.

Overdose/Acute Toxicity

There are no known antagonists for butyrophenone drugs and treatment for overdoses should focus on supportive care. Patients should be monitored for hypotension and respiratory depression and treated accordingly.

Regulatory Information

Controlled drug status: Azaperone in a nonscheduled drug.

Withdrawal times: FARAD has no withdrawal times listed for azaperone in pigs. However, a 10-day withholding period prior to slaughter has been recommended.

Veterinary Approved Products (Representative)

Azaperone: Stresnil, Azaperone 40 mg/ml (Elanco, Eli Lilly).

Benzodiazepine Derivatives

General Pharmacological Considerations

History/ Introduction

The first benzodiazepine, chlordiazepoxide, was discovered accidently in 1954 by Dr Leo Sternbach. Diazepam (Valium$^{®}$) is a simplified version of chlordiazepoxide and was marketed in 1963 for anxiety. Although many benzodiazepines (>50) are used in people and animals for behavioral modification, only diazepam, midazolam, lorazepam, and zolazepam will be discussed as sedatives and adjuncts to veterinary anesthesia. The use of benzodiazepines for managing behavior problems in animals is discussed in Chapter 18.

Classification

Benzodiazepines are sedative-hypnotics due of their propensity to cause anxiolysis, sedation, and an ability to cause sleep. They are classified as minor tranquilizers.

Chemistry: A benzodiazepine consists of a benzene ring which is fused to a seven-member diazepine ring (Figure 14.2). All of the clinically useful benzodiazepines and most of the active metabolites contain an aryl substitute at the 5th position of the diazepine ring as well as N-substitutions at the 1st and 4th positions (5-aryl-1,4-benzodiazepine). The hypnotic-sedative action of these drugs is due to the substitution of a halogen or a nitro group at the 7th position of the benzodiazepine structure.

Mechanism of Action

Benzodiazepines bind to the benzodiazepine receptor binding site on the gamma subunit of the gamma-aminobutyric acid receptor subtype A ($GABA_A$). Gamma-aminobutyric acid (GABA) is the primary inhibitor neurotransmitter in the CNS. Activation of the benzodiazepine receptor enhances the effects of GABA on the $GABA_A$ receptor. $GABA_A$ is a large macromolecule, which also contains a number of binding sites for other sedative drug classes such as barbiturates and alcohols. This explains the synergistic effect of these drugs on $GABA_A$-mediated inhibition of the CNS. Activation of the benzodiazepine binding site on $GABA_A$ receptors increase the frequency of the opening of the chloride ion channel, leading to hyperpolarization of the postsynaptic neuron (Yeh et al., 1988), producing decreased neuronal transmission. The heterogeneity of the $GABA_A$ subunits is in part responsible for the differing clinical actions of the various benzodiazepines (Upton et al., 2001). The highest concentration of $GABA_A$ receptors is found in the cerebral cortex, with very few receptor sites found outside the CNS, hence the minimal cardiopulmonary effects of benzodiazepine drugs (Cornick-Seahorn and Seahorn, 1998).

Indications

Benzodiazepines are used in veterinary medicine as: anticonvulsants (Chapter 17); adjuncts to anesthetic induction agents; skeletal muscle relaxants; and for behavioral modification (anxiolysis and sedation) (Chapter 18). In the healthy patient, behavioral effects are mild, and may be paradoxical as the reduction in inhibitions can result in vocalization, excitement, and dysphoria. Sedation is more reliable in neonates, geriatrics, or ill patients, or as the author would say “very young, very old, or very sick.” In small ruminants benzodiazepines are generally effective at causing sternal recumbency and sedation.

Pharmacodynamic effects

Central nervous system effects: A common use for benzodiazepines is to produce sedation; however, when used alone they can cause unpredictable results. This is especially true in healthy individuals. Paradoxical excitement, agitation, vocalization, and dysphoria may be seen after IV or IM administration. Benzodiazepines are commonly combined with other agents such as opioids, α_2-adrenergic agonists (see Section Alpha-2-Adrenergic Receptor Agonists), or NMDA receptor antagonists (e.g., ketamine) to provide a more predictable sedation or chemical restraint. Benzodiazepines can by anxiolytic (hence their use in people to treat phobias), but the decrease in inhibitions can result in unpredictable behavior in veterinary patients. The CNS depression produced by benzodiazepines can reduce the requirements for anesthetic induction agents and lower the inhalant needs. Diazepam potentiates the opioid reduction in the MAC of inhalant anesthesia (Hellyer et al., 2001; Seddighi et al., 2011).

Benzodiazepines have a favorable profile with regard to cerebral perfusion, which and makes them useful drugs in the face of central CNS disease. They cause a reduction in cerebral blood flow and an even greater reduction in oxygen consumption (Reves et al., 1985; Hoffman et al., 1986). Diazepam caused a reduction in theta, delta, alpha, and beta-frequencies of the electroencephalogram of anesthetized dogs without any changes to the cardiovascular parameters (Court and Greenblatt, 1992). Diazepam, like other benzodiazepines is anticonvulsant (Wauquier et al., 1979; Podell, 1995, 1996). Anticonvulsants are discussed further in Chapter 17.

Cardiovascular effects: Clinical doses of benzodiazepines cause minimal cardiovascular depression (Jones et al., 1979) and are commonly administered to patients with cardiovascular disease (Harvey and Ettinger, 2007). At clinical doses, both midazolam and diazepam have minimal effect on cardiac output, stroke volume, systemic vascular resistance, or coronary blood flow (Jones et al., 1979).

Respiratory effects: At clinical doses, benzodiazepines may decrease respiratory rate, but rarely affect ventilation and oxygenation (McDonell and Kerr, 2007). However, at higher doses, there can be a dose-dependent respiratory depression and any respiratory depression may be exaggerated when benzodiazepines are combined with other CNS depressants or when administered to debilitated patients (McDonell and Kerr, 2007).

Musculoskeletal effects: Benzodiazepines potentiate the GABA-ergic mediated muscle relaxation of inhibitory neurons at the level of the spinal cord (Elliott,

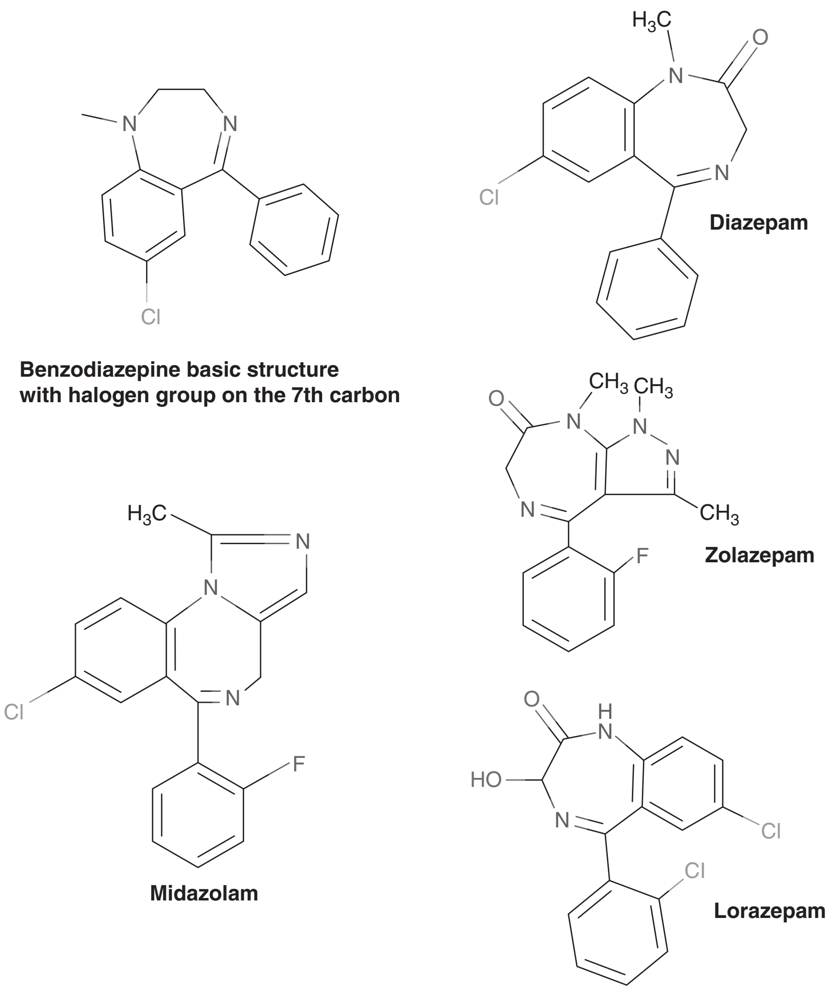


Figure 14.3 Benzodiazepines.

1976), which produces reliable muscle relaxation. Benzodiazepines are often administered with other anesthetic drugs that do not provide sufficient muscle relaxation alone (e.g., ketamine, etomidate; see Chapter 12). At the clinical doses, midazolam can cause ataxia and in some species is capable of causing recumbency (Platt et al., 2000). The effects of benzodiazepines with nondepolarizing neuromuscular antagonists are unclear. In an in vitro study using cat muscle, midazolam and diazepam were shown to potentiate neuromuscular blockade (Driessen et al., 1987a); however, in humans, midazolam was not shown to change the blockade or rocuronium (Hepağuşlar et al., 2002).

Analgesia: Benzodiazepines do not affect the pain pathway and therefore do not produce analgesia.

Specific Drugs

Diazepam Hydrochloride

Synonyms: Valium, Apozepam.

Molecular formula: C16-H13-Cl-N2-O (Figure 14.3).

Molecular weight: 284.7447.

Chemical name: 2H-1,4-Benzodiazepin-2-one, 7-chloro-1,3-dihydro-1-methyl-5-phenyl.

Diazepam has been used clinically since 1963 and is often the standard against which all other benzodiazepines are measured. Diazepam is a highly lipid-soluble benzodiazepine which has a much shorter duration of action in the dog and cats compared with horses or people (Table 14.1).

Many of the unwanted side effects of diazepam are caused by the hyperosmotic vehicle, propylene glycol (see below in this section) and therefore large volumes or constant rate infusions should be administered with caution. It may not be compatible with some aqueous IV fluids. Additionally, propylene glycol can result in erratic absorption with any route other than IV (Divoll et al., 1983).

Diazepam can administered rectally and is available in suppository form as an anticonvulsant for children

Table 14.1 Benzodiazepine pharmacokinetics in various species

Drug	Species	Dose (mg)	Volume of distribution (l/kg)	Protein binding (%)	Clearance (ml/kg/min)	Elimination half-life (minutes)	Reference
Diazepam	Dog	0.5 mg/kg IV				14–16	(Papich and Alcorn, 1995)
	Dog	2.0 mg/kg IV				192	(Löscher and Frey, 1981)
	Cat	5.0 mg/kg IV			4.72 ± 2.45	330	(Cotler et al., 1984)
	Horses	0.2 mg/kg IV				210–1320	(Muir et al., 1982)
Midazolam	Dog	0.5 mg/kg IV	3.0 ± 0.9	96		59–95	(Court and Greenblatt, 1992)
	Dog	0.2 mg/kg IV	1.1 ± 0.68		10.1 ± 1.9	63.3 ± 28.5	(Schwartz et al., 2013)
	Horse	0.1 mg/kg IV	2.8 (2.2–7.0)		10.4 (8.4–17.6)	408 (192–924)	(Hubbell et al., 2013)
Lorazepam	Dog	0.3 mg/kg IV	100 ± 15 l				(Podell et al., 1998)

(Knudsen, 1979). In dogs, diazepam at 2 mg/kg via rectal suppository did not result in clinically useful plasma levels of diazepam (Probst et al., 2013); however, the active benzodiazepine metabolites were produced in dogs administered the same dose, which indicates good rectal absorption (Papich and Alcorn, 1995).

Hemolysis: Propylene glycol, the vehicle of diazepam, can cause lysis of red blood cells (RBC), particularly in cats (Christopher et al., 1989). Large volumes or constant rate infusion may cause clinical hemolysis and therefore should be administered with discretion.

Pain on administration: Diazepam may cause pain when administered intravenously or intramuscularly (Olesen and Hüttel, 1980). The pain is associated with the vehicle, propylene glycol. Propylene glycol vehicles have also been associated with increased risk of thrombophlebitis (Doenicke et al., 1994). Clinical ways to decrease the pain for the patient involve administration in a larger vein or through a running fluid line to provide dilution.

Hepatic necrosis in cats: Repeated oral administration of diazepam has been associated severe life-threatening hepatic necrosis in cats (Center et al., 1996). No definitive cause has been established, but it is not recommended to use the oral preparation of diazepam in cats (Center et al., 1996).

Appetite stimulation: Diazepam administered intravenously is an appetite stimulant, particularly in cats (Mereu et al., 1976) and goats (Miert et al., 1989). The mechanism of action is unknown, but may be associated with decreased inhibitions or serotonergic mechanisms. Anorexia due to GI stasis should not be treated with diazepam since diazepam also slows down gastric emptying (Steyn et al., 1997).

Pharmacokinetic Data

See Table 14.1.

Representative Veterinary Dosage

See Box 14.3.

Box 14.3 Diazepam dosages

Species	Dose and route
Dog and cat	Sedation: 0.1–0.5 mg/kg IV. (IM administration is no longer recommended since propylene glycol is painful on IM injection and absorption is erratic (compared with IV dosing). Rectal dose: 0.2–2.0 mg/kg Adjunct induction agent: 0.1–0.2 mg/kg IV[a] Anticonvulsant: 0.5–1.0 mg/kg IV Appetite stimulant in cats: 0.05 mg/kg IV
Horses	Neonatal sedation: 0.05–0.2 mg/kg IV Adjunct to anesthesia induction: 0.05–0.1 mg/kg IV Anticonvulsant: 0.02–0.4 mg/kg IV
Small ruminants	Sedation: 0.1–1.0 mg/kg IV Adjunct to anesthesia induction: 0.05–0.3 mg/kg IV Appetite stimulation (goats): 0.06 mg/kg IV

[a] Diazepam is often used as an induction synergistic adjunct to propofol, ketamine, or etomidate at a rate of given immediately before the induction agent.

Regulatory Information

Controlled drug status: Diazepam is a DEA Schedule IV drug

Withdrawal times: None of the benzodiazepine drugs are intended for food producing animals and there are no established withdrawal times.

Veterinary Approved Products

There are no FDA veterinary approved products.

How supplied: Injectable formulations of diazepam consist of a 5 mg/ml solution with 40% propylene glycol, 10% ethanol, 5% sodium benzoate/benzoic acid buffer, and 1.5% benzyl alcohol as a preservative. The solution is buffered to a pH of 6.2–6.9. Propylene glycol and ethanol allow the diazepam to be dissolved into solution. The cloudy appearance of solutions does not alter the potency of diazepam; however, do not administer if a precipitate forms and does not clear.

Midazolam Maleate

Synonyms: Versed, Dormicum, Hypnovel, Rocam.

Molecular formula: C18-H13-Cl-F-N3 (Figure 14.3).

Molecular weight: 325.7727.

Chemical name: 4H-Imidazo(1,5-a)(1,4)benzodiazepine, 8-chloro-6-(2-fluorophenyl)-1-methyl-

Midazolam is a water-soluble benzodiazepine. The chemical structure of midazolam produces both water and lipid solubility based on pH (Kanto, 1985). The pH of marketed midazolam is acidic (pH = 3.5). The low pH stabilizes the diazepine ring and increases water solubility. After administration the pH increases to >4.0 (physiological pH), which causes the diazepine ring to open, increasing the lipid solubility (Kanto, 1985). Therefore at injection the drug is more water soluble but quickly becomes more lipid soluble once in the body.

The lack of propylene glycol allows administration IV, IM, and likely SQ, with reliable bioavailability (Schwartz et al., 2013). Midazolam can also be absorbed intranasally (Henry et al., 1998). However, unlike diazepam, midazolam administered rectally does not result in clinically useful plasma concentrations and cannot be recommended (Schwartz et al., 2013).

Midazolam is more potent than diazepam, but has similar pharmacodynamics and is often used interchangeably in the clinical setting. The fact that midazolam can be administered via a variety of routes, it does not cause pain on injection (no propylene glycol), and has a lower cost than diazepam has made midazolam more commonly used in veterinary medicine.

Pharmacokinetic Data

See Table 14.1.

Representative Veterinary Dosages

See Box 14.4.

Box 14.4 Midazolam dosages

Species	Dose and route
Dog and cat	Sedation: 0.1–0.4 mg/kg IV, IM, SQ 0.2–1.0 mg/kg intranasal Adjunct to anesthesia induction agent: 0.1–0.3 mg/kg IV Anticonvulsant: 0.5–1.0 mg/kg IV
Ruminants	Sedation: 0.1–1.0 mg/kg IV Adjunct to anesthesia induction agent: 0.1 mg/kg IV
Horse	Neonatal sedation: 0.05–0.2 mg/kg IV Adjunct to anesthesia induction: 0.05–0.1 mg/kg IV Anticonvulsant: 0.02–0.4 mg/kg IV

How Supplied

The clinical preparations of midazolam are water based (not in propylene glycol) and buffered to a pH of 3.5. Midazolam is compatible with normal saline and lactated Ringer's solution.

Regulatory Information

Controlled drug status: Midazolam is a DEA Schedule IV drug.

Withdrawal times: None of the benzodiazepine drugs are intended for food-producing animals and there are no established withdrawal times.

Veterinary Approved Products

There are no FDA veterinary approved products.

How supplied: Midazolam hydrochloride: Each ml contains midazolam hydrochloride equivalent to 1 mg or 5 mg midazolam compounded with 0.8% sodium chloride. The pH is approximately 3 (2.5 to 3.5) and is adjusted with hydrochloric acid and, if necessary, sodium hydroxide.

Lorazepam

Synonym: Ativan.

Molecular formula: C15-H10-Cl2-N2-O2.

Molecular weight: 321.162.

Chemical name: 10-chloro-2-(2-chlorophenyl)-4-hydroxy-3,6-diazabicyclo[5.4.0]undeca-2,8,10,12-tetraen-5-one (Figure 14.3).

Lorazepam is a benzodiazepine drug that can be administered orally and parenterally. In humans, lorazepam is the drug of choice for status epilepticus due to its higher binding affinity to the benzodiazepine receptor and elevated brain concentrations (Podell et al., 1998). It achieves therapeutic levels when administered IV but does not when administered rectally (Podell et al., 1998). Intramuscular bioavailability is veterinary species is unknown, but it is well absorbed in people (Wermeling et al., 2001). Injectable lorazepam is supplied in propylene glycol, which can result in pain on administration and hemolysis, similar to diazepam (Cawley, 2001). In veterinary medicine it is uncommonly used as a sedative or anticonvulsant, but its oral bioavailability makes it suitable as an anxiolytic (Sherman, 2008).

Representative Veterinary Dosages

Dog for anxiolytic effects: 0.02–0.1 mg/kg PO BID (Sherman, 2008).

Regulatory Information

Controlled drug status: Lorazepam is a DEA Schedule IV drug.

Withdrawal times: None of the benzodiazepine drugs are intended for food-producing animals and there are no established withdrawal times.

Veterinary Approved Products

There are no FDA veterinary approved products

How supplied: The vehicle of lorazepam consists of propylene glycol 79%, polyethylene glycol 18–20%, and benzyl alcohol 2% (v/w %). Each ml contains 2 mg or 4 mg.

Zolazepam

Synonym: zolazepam HCL.

Molecular formula: C15-H15-F-N4-O (Figure 14.3).

Molecular weight: 286.3085.

Chemical name: Pyrazolo(3,4-e)(1,4)diazepin-7(1H)-one, 4-(2-fluorophenyl)-6,8-dihydro-1,3,8-trimethyl-

Zolazepam is also a water-soluble benzodiazepine, but at acid and physiological pH. In the USA, it is only available in combination with tiletamine, an NMDA antagonist (like ketamine), in the anesthetic drug Telazol® (see Chapter 12 for additional information). Little clinical information exists on zolazepam alone.

Representative Veterinary Dosages

Zolazepam is only available as part of the mixture of Telazol (see Chapter 12 for dosing of that product).

Regulatory information

Controlled drug status: Zolazepam is a DEA Schedule III drug. Note, zolazepam is only available as part of the combination drug Telazol, which is a DEA schedule III drug.

Withdrawal times: None of the benzodiazepine drugs are intended for food-producing animals and there are no established withdrawal times.

Veterinary Approved Products

Zolazepam as part of the combination product Telazol® is the only FDA-approved benzodiazepines for us in dogs and cats.

How supplied: Zolazepam is supplied only with tiletamine as a powder that is reconstituted to 5 ml of solution. Each ml contains 50 mg zolazepam (and 50 mg tiletamine). The reconstituted solution has a pH of 2.2–2.8.

Species Differences

Cat: There have been case reports of cats developing hepatic failure following repeated oral administration of diazepam (see Section Diazepam Hydrochloride). Diazepam is an appetite stimulant, particularly in cats (see Section Diazepam Hydrochloride).

Horses: In adult horses, and depending on dose, midazolam may not produce sedation (Hubbell et al., 2013) or can cause pacing, paddling, and agitation (Löscher and Frey, 1984). Benzodiazepines are rarely used alone in adult horses for sedation.

Zolazepam and veterinary species: (See also Chapter 12.) Zolazepam is metabolized at different rates in different species. This is clinically relevant as it is combined with tiletamine in the product Telazol. Therefore recovery (time and smoothness) from Telazol can be very different in different species.

Cerebral uptake of benzodiazepines: There are some species differences in the cerebral uptake of benzodiazepines in various species. In cats and sheep, midazolam is taken up by the cerebrum faster than diazepam; however, the converse is true for pigs (Upton et al., 2001). This is in part due to the lower brain : blood partition coefficient of midazolam when compared with diazepam. In dogs, the free unbound portion of the benzodiazepines correlates well with the concentration of these corresponding compounds to brain tissue levels (Wala et al., 1991).

Metabolism

Benzodiazepines undergo liver metabolism, but the pathways (e.g., reduction, glucuronidation) differ based on the drug and species. Additionally, many of the benzodiazepines have a number of have active metabolites such as desmethyldiazepam, oxazepam, and temazepam (Upton et al., 2001), which also varies by drug and species. Desmethyldiazepam is the main metabolite in most species (Wala et al., 1991), and appears to have the most clinical effect due to its long half-life. Approximately 50% of diazepam is metabolized to desmethyldiazepam in the cat (Cotler et al., 1984). Following glucuronide conjugation benzodiazepines are excreted in the urine (Martin et al., 1990); however, cats perform glucuronidation much slower than other species (Driessen et al., 1987b).

Adverse Effects/ Contraindications

Paradoxical excitation

It is not uncommon to see a transient period of agitation, vocalization, or excitement following parenteral benzodiazepine administration in a number of species. This unwanted effect is seen more commonly in healthy animals, and less commonly in very young, very old, or ill patients. Since, benzodiazepine drugs when used alone can produce unpredictable behavioral effects, they are often administered in conjunction with other CNS depressants to minimize the risk of paradoxical excitation.

Behavioral disinhibition

As an anxiolytic, benzodiazepines can decrease inhibitions. Therefore caution should be used when sedating animals exhibiting fear-induced aggression, as the disinhibition caused by the benzodiazepines may provoke aggressive behavior.

Hepatic Necrosis in Cats with Oral Diazepam

See Section Diazepam Hydrochloride.

Addiction/Physical Dependence

Patients discontinuing a benzodiazepine require their dose to be tapered off to avoid abstinence syndrome from sudden cessation. Long-term use of benzodiazepines does cause physical dependence in the dog (McNicholas et al., 1983) and the use of flumazenil may precipitate abstinence syndrome (tremors, hot foot walking twitches, tonic clonic seizures, and occasional death) (Oliver et al., 2000; Klotz, 1988).

Drug Interactions

The administration of other CNS depressants (e.g., inhalant anesthetics, propofol, opioids, α_2-adrenergic agonists) with a benzodiazepine often results in an additive or synergistic CNS depressant effect (Short and Chui, 1991). Therefore dosing of benzodiazepine or other drugs may need to be adjusted.

Diazepam is absorbed by the polyvinylchloride (PVC) materials commonly used in IV solution lines, bags, and syringes (Kowaluk et al., 1983; Daniell, 1975), with up to 50% of the drug being absorbed (Ball and Tisocki, 1999). This can be clinically relevant for patients receiving constant rate infusions or if storing diazepam in administration sets. This binding does not occur with hard plastic (e.g., syringes) when used for administration.

Benzodiazepines that first undergo oxidation as part of their hepatic metabolism, such as diazepam or midazolam, may have reduced metabolism if coadministered with cimetidine, erythromycin, isoniazid, ketoconazole (KuKanich and Hubin, 2010), propranolol and valproic acid, whereas rifampin will increase the metabolic rate of benzodiazepines (Lam et al., 2003).

Overdose/Acute Toxicity

Clinical signs of acute intoxication can include ataxia/disorientation, CNS depression or agitation, respiratory depression, weakness, tremors, vocalization, tachycardia, tachypnea, or hypothermia. Treatment should include the use of the specific benzodiazepine antagonist, flumazenil (see Section Flumazenil), and supportive care.

Benzodiazepine Antagonists

General Pharmacological Considerations

History/ Introduction

Flumazenil was introduced in 1987 by Hoffmann-La Roche under the trade name Anexate^{TM}. It is the only benzodiazepine antagonist available in the USA. It has also been marketed under the trade name Romazicon, but is currently used as a generic.

Classification

Flumazenil is a specific and exclusive benzodiazepine competitive antagonist with a high affinity for the benzodiazepine receptor site of the $GABA_A$ receptor. Flumazenil has virtually no agonist activity at the benzodiazepine receptor site.

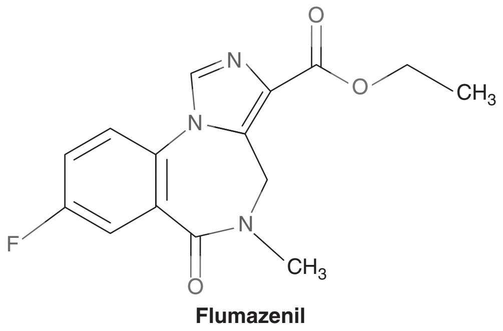


Figure 14.4 Flumazenil.

Specific Drugs

Flumazenil

Molecular formula: C15-H14-F-N3-O3 (Figure 14.4).

Molecular weight: 303.3 g/mol.

Systematic chemical name: 4H-Imidazo(1,5-a)(1,4) benzodiazepine-3-carboxylic acid, 8-fluoro-5,6-dihydro-5-methyl-6-oxo-, ethyl ester.

Mechanism of Action

Flumazenil competitively antagonizes the action of benzodiazepines on the benzodiazepine receptor binding site on the $GABA_A$ receptor. Activation of the $GABA_A$ receptor results in increased chloride conduction and subsequent hyperpolarization of the postsynaptic membrane (decreased neural conduction). Antagonism of this effect results in more active neural conduction. Flumazenil only binds and antagonizes the benzodiazepine binding site of the GABA receptor. Therefore, it does not antagonize the CNS effects of other $GABA_A$ receptor sedative-hypnotics at such as propofol or ethanol. Flumazenil antagonizes endogenous benzodiazepine-like substances, which are elevated in human patients with hepatic encephalopathy (Grimm et al., 1988).

Indications

Flumazenil is indicated for the competitive reversal of benzodiazepine agonists.

Pharmacodynamic Effects

Central nervous system effects: Flumazenil attenuates the CNS depressant effects of benzodiazepine drugs. Flumazenil reverses the electroencephalographic changes induced by benzodiazepine in the dog and horse (Greene et al., 1992; Keegan et al., 1993; Johnson et al., 2003; Artru, 1989). In people, flumazenil in the absence of a benzodiazepine did not cause CNS excitement (Forster et al., 1993).

The benzodiazepine antagonist, sarmazenil, has been shown to be useful in the treatment of hepatic encephalopathy (Grimm et al., 1988); however, flumazenil has been shown minimal effect in reversing the effects of hepatic encephalopathy (Grimm et al., 1988; Meyer et al., 1998).

Cardiovascular effects: Flumazenil has no direct effect on left ventricular function or coronary hemodynamics observed in human patients (Marty et al., 1991); however, in cats flumazenil administered after diazepam or midazolam antagonized the fall in blood pressure caused by the benzodiazepines agonists (Driessen et al., 1987c).

Musculoskeletal effects: Flumazenil is effective in reversing the muscle relaxation associated with the benzodiazepine agonists.

Respiratory effects: Tidal volume and minute ventilation are generally restored to normal with the use of flumazenil following a benzodiazepine; however, the CO_2 response curve of the respiratory center may still depressed (Shalansky et al., 1993). Flumazenil in the absence of a benzodiazepine does not exhibit any respiratory stimulatory effects even at ten times the dose in people (Forster et al., 1993).

Analgesia: Activating or blocking the $GABA_A$ receptor should not affect the pain pathway.

Miscellaneous: Flumazenil does not affect intraocular pressure in health human volunteers when given alone, but does reverse the decrease in intraocular pressure seen after the administration of benzodiazepines (Artru, 1991).

Adverse effects/ contraindications

The use of flumazenil in patients who have been treated chronically with benzodiazepine or have received an overdose of tricyclic antidepressants has the potential of precipitating seizures (Spivey, 1992). This phenomenon of benzodiazepine abstinence syndrome has been seen in dogs experimentally (Lheureux et al., 1992).

To help reduce the pain or discomfort at the injection site, flumazenil may be diluted or administered into a large vein. Extravasation of flumazenil may result in local tissue inflammation and necrosis (Smith and Volmer, 2005).

Metabolism

There is limited information in veterinary species, but in people flumazenil is extensively metabolized by the liver

(99%) to the deethylated free acid and glucuronide conjugate (Klotz, 1988; Schlappi et al., 1988), both of which are excreted in the urine. At least three metabolites have been identified, but none of the metabolites of flumazenil appear to be active (Oliver et al., 2000).

Pharmacokinetics Properties

Although there is pharmacodynamic evidence that flumazenil is effective in veterinary species, there is limited pharmacokinetics information. In humans, the rapid onset of action is due to a rapid uptake and distribution (Klotz and Kanto, 1988). Pharmacokinetic profile in people includes: half-life of 0.7–1.3 hours; apparent distribution volume 0.6–1.6 l/kg; blood clearance of 520–1300 ml/min; and a plasma protein binding of flumazenil of about 40% (Klotz and Kanto, 1988). In dogs, the time for reversal in a model using midazolam-induced respiratory depression with flumazenil was 120 ± 25 seconds IV and up to 310 ± 134 seconds IM (Heniff et al., 1997). Due to the extensive liver metabolism, hepatic diseases can lengthen the elimination phase.

Multiple routes of administration are effective for flumazenil in the dog (IV, IM, sublingual, and per rectal), although the IV route produces the fastest effect (Heniff et al., 1997). Endotracheal administration is also effective in people (Palmer et al., 1998). Despite the fact that flumazenil has a high first-pass effect and the oral route is not recommended (Smith and Volmer, 2005), in dogs it is absorbed after oral administration with peak levels at 1 hour and lasting 4 hours (Wala et al., 1988). However, since benzodiazepine antagonists are generally given due to excessive sedation or side effect, the oral route many not be clinically useful.

Doses

In dogs the dose for benzodiazepine reversal is 0.01–0.04 mg/kg IV, IM, sublingual, endotracheal, or rectal. Repeated doses of flumazenil may be required for the reversal of a benzodiazepine-induced sedation.

In horses, the dose for benzodiazepine reversal is 0.01–0.02 mg/kg IV (Kaegi, 1990; Cornick-Seahorn and Seahorn, 1998).

Regulatory Information

Controlled drug status: Flumazenil in not an FDA scheduled drug.

Withdrawal times: No withdrawal times have been formulated for flumazenil.

Veterinary Approved Products

There are no FDA veterinary approved products.

Veterinary Approved Products Intravenous preparations of flumazenil are 0.1 mg/ml in a 5-ml vial. Each ml contains 0.1 mg of flumazenil and is compounded with 1.8 mg of methylparaben, 0.2 mg of propylparaben, 0.9% sodium chloride, 0.01% edetate disodium, and 0.01% acetic acid. The pH is adjusted to 4 using hydrochloric acid and/or sodium hydroxide.

Overdose/Acute Toxicity

There is limited information on overdoses in veterinary species. However, administration of flumazenil in the absence of benzodiazepines has not produced CNS excitement or tachypnea. Complications associated with accidental overdose or idiosyncratic reaction might be successfully treated with either a benzodiazepine or $GABA_A$ agonist.

Alpha-2-Adrenergic Receptor Agonists

Introduction

Alpha-2-adrenergic agonist drugs are popular in veterinary medicine because they produce profound, reliable sedation as well as analgesia. The first α_2-adrenergic agonist drug used in veterinary medicine was xylazine. It was developed in Germany in 1962 as a human antihypertensive drug (Greene and Thurmon, 1988) but clinical evaluation identified sedation as a potent side effect and the drug was marketed to veterinarians for that use. The veterinary use of α_2-adrenergic agonists was first reported in the late 1960s (Clarke and Hall, 1969), which revolutionized sedation and anesthesia, particularly in large animal patients. Furthermore, α_2-adrenergic agonists act synergistically with opioids so are often used as rescue analgesics. Administration of α_2-agonists can be parenteral, intravenous, intramuscular, and subcutaneous, as well as by transmucosal, transdermal, and neuraxial routes. This class of drug also popular because the effects can be reversed with the use of α_2-adrenergic antagonist drugs (see Section Alpha-**2**-Adrenergic Antagonists).

Classification

The pharmacology of adrenergic agonists and antagonists is covered in Chapter 7. Information on basic pharmacology can be found in that chapter. α_2-adrenergic agonists are sedatives that also possess significant analgesic properties. Although described as α_2-adrenergic receptor agonists, all of the drugs in this category have some agonist effect on α_1-adrenergic receptors, and some will bind and activate imidazole receptors.

Table 14.2 Ratio of drug selectivity and imidazoline receptor activity

Compound	$\alpha_2 : \alpha_1$ selectivity	I_2-Imidazoline activity
Agonists		
Xylazine	160	No
Detomidine	260	Yes
Romifidine	340	Yes
Medetomidine	1620	Yes
Dexmedetomidine	1620	Yes
Antagonists		
Tolazoline		Yes
Yohimbine	40	No
Atipamezole	8526	No

Chemistry

Alpha-2-receptor agonist drugs differ clinically primarily by their differing affinities for α_2 or α_1 adrenergic receptors, also referred to as the $\alpha_2 : \alpha_1$ ratio (Table 14.2). Drugs that are more specific for the α_2-receptor will have a high ratio and the drugs that are less specific for the α_2-receptor will have a lower ratio.

How Supplied

Most α_2-adrenergic agonists used in veterinary medicine are administered parenterally and are dispensed in varying concentrations (see Section Veterinary Approved Products). The veterinary exception is a newly marketed transmucosal gel marketed for horses. The human product, clonidine, is available in oral, ophthalmic, and transdermal patch formulations. Dexmedetomidine is also approved as a human injection (Precedex).

Mechanism of Action

Alpha-2-adrenergic receptors are G-protein receptors and are located both pre- and postsynaptically in the CNS and peripherally. α_2-adrenergic receptors are also located extrasynaptically on the vascular endothelium of vessels as well as on platelets. Furthermore, α_2-adrenergic receptors have been divided into four subtypes (α_{2A}, α_{2B}, α_{2C}, and α_{2D}). Humans express only subtype A–C, whereas some domestic animals, such as cattle, express the D subtype. The presence and percentage of subtypes varies with species, which likely explains the different pharmacodynamic effects and susceptibility among difference species. In general, the α_{2A} subtype is associated with arousal and awareness in the brainstem and the α_{2B} subtype is associated with mediating vasoconstriction. Cattle have a higher percentage of α_{2D} subtypes, which also appear associated with arousal. Although it has been demonstrated that activation of particular subtypes results in different physiological responses (e.g., analgesia or vasoconstriction), none of the current drugs are subtype specific.

The sedative effects of α_2-agonists are mediated by presynaptic binding of α_2-adrenergic receptors supraspinally, in the locus ceruleus of the pons (Scheinin and Schwinn, 1992). Binding of these presynaptic receptors results in a decrease synaptic release of norepinephrine (NE) (Cormack et al., 2005). Norepinephrine is the primary neurotransmitter of the sympathetic nervous system and decrease in NE results in decreased neurotransmission and decreased arousal (sedation).

The analgesic effects of α_2-agonists are primarily mediated in the dorsal horn of the spinal cord via decreased release of NE and substance P. However, there is likely also some descending modulation of nociceptive input via the locus ceruleus (Hellyer et al., 2007).

The α_2-adrenergic agonists also have agonist activity on α_1 and α_2-adrenergic receptors found in vessel vasculature (primarily α_1). Activation of these receptors results in increase in systemic vascular resistance via vasoconstriction of vessels.

The α_2-adrenergic agonists also bind at nonadrenergic imidazoline receptors (Khan et al., 1999). There are at least three subtypes of imidazoline receptors. Imidazoline-1 are associated with central blood pressure control and may contribute to the drugs cardiovascular effects, although the extent and significance is yet to be fully understood (Khan et al., 1999). Additionally, activation of imidazoline-2 receptors may contribute to the analgesic effects (Head and Mayorov, 2006).

Indications

The α_2-adrenergic agonist drugs are used in veterinary patients to provide sedation, chemical restraint, analgesia, and as an adjunct to anesthetics. Some α_2-agonists are also used as emetics, particularly in cats (see Section Gastrointestinal Effects).

Physiological Effects

Central Nervous System Effects

Alpha-2-adrenergic receptors produce profound, reliable sedation in most veterinary species (see Section Mechanism of Action). However, there are differences in response based on species, drug, and dose. The pharmacodynamic differences between species are likely caused by differences in the α_2-adrenergic receptor subtypes in the CNS between species, most notably the presence of α_{2D}-adrenergic receptors in ruminants (Schwartz and Clark, 1998). Ruminants in particular are very sensitive to the sedative effects of certain α_2-adrenergic agonists (e.g., xylazine) in the locus coeruleus nucleus (Scheinin and Schwinn, 1992). Therefore, it is likely that the population of α_{2D}-receptor subtypes in the ruminant CNS is different than other animals.

The duration and depth of the sedation are dose dependent. Lower doses generally produce mild to moderate sedation, where high doses can produce unconsciousness in some species, such as dogs (Kuusela et al., 2001a, 2003). There does appear to be a ceiling effect for sedation in some species, such as horses, where increased doses generally do not result in recumbency, but rather increases the duration of effect.

All currently available α_2-agonist drugs have some α_1 agonist properties. Activation of only α_1-adrenergic receptors in the CNS can cause arousal, agitation, increased locomotor activity, and vigilance (Sinclair, 2003). While excitation is rarely seen, the lack of α_1 activation may contribute to why more selective α_2-agonist drugs generally produce more reliable sedation (see Table 14.2). Rarely, and surprisingly, sedation with α_2-agonists (particularly xylazine, which has the most α_1 effect) in horses can result in aggression (Hubbell and Muir, 2004).

The CNS depression (and analgesia) associated with α_2-agonists produce profound anesthetic-sparing effects (Kuusela et al., 2003; Kauppila et al., 1992; Savola et al., 1991) of both induction agents and MAC sparing of up to 90% (Vickery et al., 1988). It is therefore imperative when using these agents as part of multimodal anesthesia to monitor and adjust other drug doses accordingly.

Intracranial pressure was unchanged in dogs anesthetized with medetomidine (Keegan et al., 1995). Dexmedetomidine in anesthetized dogs was associated with a decrease in cerebral blood flow; however, there was no evidence of ischemia even with the reduced cerebral blood flow (Zornow et al., 1990). Although α_2-agonists are not associated with increased intracranial pressure and cerebral perfusion was maintained, considerations must include the expected decrease in cardiac output, increase in systemic vascular resistance, as well as the potential for hyperglycemia (see Section Glucose). Furthermore, the high incidence of emesis in susceptible small animals also needs to be considered when used in the preoperative period in animals with elevated intracranial pressure. The use of α_2-agonists in patients with CNS disease needs to be assessed on a case to case basis (Cormack et al., 2005).

Cardiovascular Effects

Alpha-2-adrenergic agonists produce a biphasic cardiovascular response. In the "first" phase, activation of presynaptic central α_2-adrenergic receptors reduce the sympathetic outflow (decreased norepinephrine) and thereby increases the parasympathetic tone, which will result in negative inotropic, chronotropic, and dromotropic effects on the heart as well as peripheral vasodilation. High vagal tone and decreased dromotropy often causes first and second-degree atrioventricular heart block. Peripherally, postsynaptic α_2 and α_1-adrenergic receptors are activated in the vascular endothelium causing profound vasoconstriction (which overshadows the vasodilation from the lack of norepinephrine). The increase in arterial blood pressure results in a baroreceptor-mediated reflex bradycardia. Most patients in this first phase are hypertensive and bradycardic. During the "second" phase there is a decrease in systemic vascular resistance (less activation of peripheral vascular adrenergic receptors); however, the heart rate remains low (likely due to decreased norepinephrine). Patients during the second phase may be hypotensive and bradycardic.

At therapeutic doses, α_2-agonists decrease cardiac output in most species by more than 50% (Pypendop and Verstegen, 1998; Lamont et al., 2001; Bueno et al., 1999). Reduction in cardiac output is not only due to the baroreceptor reflex but concomitant reduction in stroke volume, increase afterload, and low catecholamine levels. There appears to be a ceiling effect with the cardiovascular responses to α_2-adrenergic agonists (Kuusela et al., 2000; Pypendop and Verstegen, 1998, 1999). Higher doses produce more sedation, analgesia, and longer duration of action, but do not necessarily worsen the cardiovascular response.

The use of anticholinergics prior to or at the same time as an α_2-adrenergic agonist is controversial. During the first phase, bradycardia is a protective baroreceptor reflex and generally should not be treated. Treatment with anticholinergics only partially prevent the decrease in cardiac output and increase the risk of dysthymia (Sinclair, 2003; Short, 1991) and hypertension (Singh et al., 1997). Hypertension is more likely when the anticholinergic is administered during the initial vasoconstrictive phase than in the secondary hypotensive phase (Pimenta et al., 2011). Arrhythmias are more likely to occur when the anticholinergic is given after the cardiovascular effects (i.e., hypertension and bradycardia) are seen compared with prior to or concurrently with an α_2-agonist (Short, 1991). Arrhythmias associated with α_2-agonists and anticholinergics are likely due to increases in myocardial workload (increased heart rate and increased afterload) and oxygen consumption. α_1-adrenergic receptors have also been implicated in the formation of arrhythmias mediated via α_2-adrenergic agonists, especially with xylazine due to its relatively low $\alpha_2 : \alpha_1$-adrenergic receptor affinity (Bozdogan and Dogan, 1999).

Alpha-2-adrenergic agonists will redistribute blood flow from nonessential regions such as the skin and viscera to the central organs like the brain, heart, and kidneys (Pypendop and Verstegen, 1998; Lawrence et al., 1996). Furthermore, they do not decrease coronary artery perfusion or myocardial oxygenation (Snapir et al., 2006). This may explain why a class of drugs with so many cardiac effects has been shown to improve survivability in human patients undergoing cardiovascular surgery (Wijeysundera et al., 2003).

Respiratory Effects

Alpha-2-adrenergic agonists tend to cause a centrally mediated reduction in respiratory rate and minute ventilation, with no or with mild increases in Paco_2 and mild reductions in Pao_2 (Kolliasbaker et al., 1993; Pypendop and Verstegen, 1999; Sinclair, 2003; Lerche and Muir, 2004). The respiratory depression is not as great compared to other anesthetic drugs such as opioids (Sinclair, 2003) or inhalational anesthetics (Bloor et al., 1989). Respiratory depression is exaggerated with the addition of other respiratory depressants or CNS depressants, which can result in hypercapnia, hypoxemia, and cyanosis (Pypendop and Verstegen, 1999). It is speculated that cyanosis of mouth and gums may also be due to slower blood flow through the peripheral capillary beds and an increased oxygen extraction (Sinclair, 2003). It is therefore recommended to monitor patients for hypoventilation and hypoxemia and administer supplemental oxygen as needed, especially in those receiving a combination of respiratory depressants.

Serious, species-specific, respiratory side effects caused by α_2-adrenergic agonists can occur (Bloor et al., 1989; Celly et al., 1997). The most noted complication occurs in sheep. Within minutes of α_2-adrenergic agonist administration in sheep there can be activation of pulmonary intravascular macrophages (PIM) that produce extensive damage to the capillary endothelium and alveolar type I cells, intraalveolar hemorrhage, and interstitial and alveolar edema (Celly et al., 1997). Pulmonary edema causes decreased alveolar gas exchange, an increase in respiratory rate and airway pressures, and a decrease in pulmonary compliance. Hypoxemia is frequently observed and some sheep will die (Celly et al., 1997). While many animal species (e.g., horses) have PIMs, the activation of these by α_2-agonists in sheep is more commonly seen clinically. Dexmedetomidine causes similar pulmonary changes in the sheep and goat (Kutter et al., 2006). Treatment with an α_2-adrenergic antagonists appears to prevent further activation of PIMs, reverses sedation, and many animals improve clinically. However, the pulmonary effects are not completely eliminated with reversal.

Musculoskeletal Effects

Alpha-2-adrenergic agonists produce reliable muscle relaxation, which is mediated via their interaction with the interneurons in the spinal cord (Sinclair, 2003). They are frequently administered for this effect and to balance drugs that do not provide good muscle relaxation (e.g., ketamine). Muscle twitching has been noted in the dog (Sinclair, 2003) and head bobbing and facial twitching has also been observed in the horse (Lloyd Industries, 2014).

Alpha-2-agonist drugs can effect muscle tone and contraction of smooth muscle such as the uterus and GI tract (see Sections Gastrointestinal Effects and Reproductive Effects).

Gastrointestinal Effects

Emesis: Alpha-2-adrenergic agonist (xylazine) may cause vomiting in up to 90% of cats and 30% of dogs (Cullen, 1999). It has been theorized that α_2-agonists interact with the chemoreceptor zone located in the area postrema to stimulate dopamine and norepinephrine receptors to cause emesis. This appears to be a postsynaptic α_2-adrenergic receptor mediated event, which can be antagonized by α_2-antagonist drugs (Jovanovic-Micic et al., 1995).

Motility and acid secretion: Alpha-2-agonists decrease gastrointestinal motility, prolong intestinal transit time, and inhibit colonic motility in a number of species, with the large bowel more sensitive to the effects of α_2-adrenergic agonists in ruminants, dogs, and horses (Sasaki et al., 2000; Maugeri et al., 1994). The depression in gastrointestinal motility can be attenuated with the use of an α_2-antagonist. α_2-agonists also decrease gastric acid secretions. The effects on intestinal motility must be considered when these drugs are used to sedate animals undergoing diagnostic tests of intestinal function. This should also be considered when administered to animals prone to complications from ileus (e.g., horses).

Renal effects

Alpha-2-adrenergic agonists cause diuresis by multiple mechanisms. They reduce the production or release of antidiuretic hormone (ADH, arginine vasopressin) from the pituitary (Humphreys et al., 1975; Reid et al., 1979); inhibit the actions of ADH on the collecting tubules; and enhance the excretion of sodium (Gellai and Edwards, 1988; Smyth et al., 1985). Renin levels are decreased by the direct activation of renal α_2-adrenergic receptors and indirectly by the initial hypertension produced by the α_2-adrenergic agonists (Smyth et al., 1987) further contributing to diuresis.

Alpha-2-adrenergic agonists also affect micturition by decreasing micturition pressure, bladder capacity, micturition volume, and residual volume via both spinal and peripherally mediated mechanism (Ishizuka et al., 1996). This, combined with diuresis, will cause animals treated with α_2-agonists to produce large amounts of dilute urine that is frequently voided.

Neuroendocrine Stress Response

Predicting the effects of α_2-adrenergic agonists on catecholamines and cortisol levels is difficult. In general, α_2-adrenergic agonists will decrease catecholamine due to their presynaptic effects on the release of norepinephrine. However, the effects on basal or induced cortisol varies based on drug, dose, and species. In the horse, detomidine will suppress catecholamine activity without suppressing cortisol activity (Raekallio, 1991) whereas in cattle, medetomidine will suppress catecholamine activity without suppressing cortisol activity (Ranheim, 2000). Interestingly, in the dog, high doses (80 μg/kg) of dexmedetomidine suppressed cortisol levels as well as prevented stimulation with ACTH (Maze et al., 1991). However, at clinical doses, dexmedetomidine did not change cortisol level in dogs (Restitutti et al., 2012).

Glucose

The effect of α_2-adrenergic agonists on glucose homeostasis is complex and dependent upon the drug, the dose, and the species. Hyperglycemia reported after the use of α_2-adrenergic agonists is likely due to a decrease in insulin release from the β-cells in the pancreas and/or increase glucagon release from the α-cells (increased gluconeogenesis) (Angel et al., 1990; Niddam et al., 1990). The α_2-adrenergic agonist, xylazine, causes a mild transient hyperglycemia in cows and horses (Thurmon et al., 1984; Hsu and Hummel, 1981). Medetomidine causes a decrease in insulin without the resultant increase in glucose in dogs (Ambrisko and Hikasa, 2003). Clinical hyperglycemia secondary to α_2-adrenergic agonist administration is generally not high enough to pass the renal threshold for glucose (~180 mg/dl) and therefore should not produce glucosuria.

Reproductive Effects

Alpha-2-agonist drugs can effect myometrial tone as well as myometrial contraction; however, these effects are related to species, dose, time of reproductive cycle, and species. Furthermore, the clinical effect of α_2-adrenergic agonists on uterine contractility and blood flow is influenced by the level of estrogen and progesterone. Estrogen leads to an up-regulation in α-adrenergic receptors while progesterone will cause a down-regulation due to changes in the transmembrane signal pathways that cause the differences in myometrial contractility (Re et al., 2002).

Myometrial contractions in the nongravid uterus were observed at any equipotent dose of α_2-adrenergic agonists (Schatzmann et al., 1994; Jedruch et al., 1989) and there is a case report of cows having premature labor after the administration of xylazine (Vanmetre, 1992). Lower doses of detomidine (<60 μg/kg IM) in the cow and horse, and medetomidine (<20 μg/kg IV) in the dog, decreased myometrial contractions (Jedruch and Gajewski, 1986; Jedruch et al., 1988, 1989). Higher doses of medetomidine in the dog did cause an increase in myometrial contraction (Jedruch et al., 1989). This biphasic response in the gravid uterus to α_2-adrenergic agonists may possibly be mediated by their effect on the α_1-adrenergic receptor (Ford, 1995; Gaspar et al., 2001; Jedruch et al., 1989).

The effects of medetomidine (40 μg/kg IM) and xylazine (200 μg/kg IM) on intrauterine pressure and uterine blood flow have been studied in the goat (Sakamoto et al., 1996, 1997). Both agents crossed the placenta, reduced uterine blood flow for 120 minutes, and increased intrauterine pressure. These observations would suggest α_2-adrenergic agents should be used with caution in near-term pregnant animals, especially if there are historical or physical evidence to suggest fetal distress is already present.

Miscellaneous

Thermoregulation: Patients may be unable to normally control body temperature in response to the administration of α_2-adrenergic agonists. This is due to the combination of CNS depression, reduction in muscle activity (reduction in shivering), and loss of vasomotor control. Clonidine and dexmedetomidine have been shown to decrease the vasoconstrictive and shivering thresholds (Talke et al., 1997). Animals may be cool to touch and unable to thermoregulate their body temperature. Care must be taken during the recovery phase to prevent both cooling and overheating of these animals.

Equine recovery: The use of α_2-adrenergic agonists during the postoperative period following inhalant anesthesia of horses has been shown to improve the quality of the recovery and reduce the number of attempts to stand, at the expense of slightly prolonging the recovery period (Bienert et al., 2003; Santos et al., 2003). The dose of α_2-adrenergic agonists used during this postanesthesia period may be as little as 10–20% of the "usual" premedication dose (Santos et al., 2003). Romifidine when used as a premedicant only exhibited a better quality of recovery as compared to xylazine (Jaugstetter et al., 2002), although recovery quality must be assessed in relation to the other anesthetic drugs administered, procedure performed, and recovery environment. The use of intraoperative constant rate infusions of medetomidine and romifidine have been shown to improve the quality of the recovery in horses with fewer attempts to stand (Kuhn et al., 2004).

Platelet activation: At very high doses, α_2-adrenergic agonists enhance catecholamine-potentiated platelet aggregation (Sjoholm et al., 1992). The clinical consequences of this reaction has not been documented.

Analgesia

Alpha-2-agonists produce profound analgesia and can be administered parenterally and neuraxially (e.g., epidurally). The first report of α_2-adrenergic agonist-induced analgesia was in 1974 evaluating clonidine as an analgesic in rats (Paalzow, 1974). Analgesia is produced by activation of α_2-receptors in the CNS in the locus coeruleus (Guo et al., 1996; Schwartz and Clark, 1998) and in the substantia gelatinosa of the dorsal horn of the spinal cord (Savola and Savola, 1996). The effects in the brain are primarily by decreased neural conduction, whereas the effects in the spinal cord are primarily through decreasing release of norepinephrine and substance P (see Section Mechanism of Action). Some of the analgesia may be mediated via I_2-imidazoline receptors (Diaz et al., 1997; Regunathan, 2006) as imidazoline receptor agonist and antagonists can modulate the analgesia produced by morphine (Gentili et al., 2006).

The analgesia produced by α_2-agonists is synergistic with opioids, lidocaine, and *N*-methyl-D-aspartate (NMDA) receptor antagonists (e.g., ketamine) (Glynn and O'Sullivan, 1996; Lee and Yaksh, 1995; Regunathan, 2006). Therefore these drugs are often used in conjunction with other analgesics.

In people with tolerance to opioids or chronic pain syndromes, α_2-adrenergic agonists are routinely used for rescue analgesia. A major limiting factor to use of this class for analgesia is the concurrent sedation and ataxia. Interestingly, higher plasma levels are needed to produce analgesia than sedation, with horses needing 10 times the plasma level to produce visceral nociception compared with plasma levels needed to produce sedation (Elfenbein et al., 2009).

Epidural administration can produce potent analgesia with minimal sedative or cardiovascular effects compared with intravenous administration (Aminkov and Pascalev, 1998; Greene et al., 1995). The primary receptor subtypes found in the spinal cord are α_{2A} and α_{2C}, but α_{2A}-receptors appears to most active in the nociceptive pathway (Stone et al., 1998). Due to the high lipophilicity of these drugs, there is some systemic absorption and systemic effect (e.g., sedation) after neuraxial administration. The use of α_2-adrenergic antagonists following neuraxially administered α_2-agonists can reduce the side effects whilst maintaining a good level of analgesia (Skarda, 1991).

Clinicians should also note that when α_2-agonist reversal agents are administered (α_2-antagonists; see Section Alpha-2-Adrenergic Antagonists), to reverse sedation, the analgesic effects will be reversed also.

Pharmacokinetics Properties

Pharmacokinetics of α_2-agonist can vary based on species, drugs, and dose. In general, the onset of action of most α_2-adrenergic agonists following intravenous administration is rapid (within minutes) and peak effect usually occurs within 10–15 minutes (Garcia-Villar et al., 1981; Pypendop and Verstegen, 1998). Intramuscular administration of α_2-adrenergic agonists will approximately double the time to peak effect; however, the bioavailability is variable (40–95%) depending upon the species (Garcia-Villar et al., 1981; Kästner et al., 2003). Bioavailability of intramuscular xylazine in the horse and sheep is approximately 50% as compared to 75% in the dog (Garcia-Villar et al., 1981). Bioavailability of intramuscular detomidine is variable depending on species: horse 66%, and cow 85%(Salonen, 1989). Bioavailability of intramuscular medetomidine is close to 100% in dogs and cats (Salonen, 1989). The pharmacokinetic parameters of the various α_2-adrenergic agonists in the common domestic species are summarized in Table 14.3.

Interestingly, there is a lack of correlation between plasma levels and pharmacodynamic effects of medetomidine in cows and sheep (Ranheim et al., 1999, 2000). Clinical effects can be seen for up to 7 hours despite the plasma levels being detectable for only 2 hours. This discrepancy implies either a long tissue binding period or an active metabolite (Ranheim et al., 1999, 2000).

Comparative Equine Doses and Effects

Equipotent intravenous doses for sedation in the horse are considered to be the following: xylazine (1 mg/kg), medetomidine (5–10 μg/kg), romifidine (40–80 μg/kg), and detomidine (20–40 μg/kg) (England et al., 1992; Yamashita et al., 2002). The order in which they are listed also demonstrates the duration of sedation from the shortest to longest in duration (Freeman and England, 2000; Hamm et al., 1995; Yamashita et al., 2002). Detomidine tends to cause the longest duration of ataxia (Hamm et al., 1995; England et al., 1992). There appears to be less ataxia and lowering of the head with romifidine as compared to the other α_2-adrenergic agonists; however, the level of analgesia achieve may be comparable to (England et al., 1992; Spadavecchia et al., 2005) or lower than (Moens et al., 2003; Hamm et al., 1995) other α_2-adrenergic agonists, depending upon the stimulus used. The apparent order of ataxia in horses produced by these agents from least to most appears to be: romifidine < xylazine < medetomidine and detomidine.

Specific Veterinary Drugs

Xylazine hydrochloride

Synonyms: Rompun AnaSed, Cervizine.

Molecular formula: C12-H16-N2-S (Figure 14.5).

Molecular weight: 220.338 g/mol.

Table 14.3 Alpha-2-adrenergic agonists pharmacokinetics

Drug	Species	Dose (mg/kg)	Mean Residence Time (minutes)	Volume of distribution (L/kg)	$\text{Clearance}_{\text{Tot}}$ (mL/kg/min)	Elimination half-life (mins)	Reference
Xyalzine							
	Dog	1.4 (IV)		2.52	81	30.1	(Garcia-Villar et al., 1981)
	Cat						
	Horse	0.6 (IV)		2.46	21	49.5	(Garcia-Villar et al., 1981)
	Cattle	0.2 (IV)		1.94	42	36.5	(Garcia-Villar et al., 1981)
	Sheep	1.0 (IV)		2.74	83	23.1	(Garcia-Villar et al., 1981)
Detomidine							
	Horse	0.08 (IV) [IM]		0.74 ± 0.25 [1.56]	7.1 ±1.6 [10.1]	71.4 ± 16.2 [106.8]	(Salonen et al., 1989)
	Cattle	0.08 (IV) [IM]		0.73 ± 0.17 [1.89]	9.5 ± 1.9 [12.3]	79.2 ± 27 [153.6]	(Salonen et al., 1989)
Medetomidine							
	Dog	0.04 (IV)		1.28 ± 0.19	21.0 ± 7.3	57 ± 15	(Kuusela et al., 2000)
	Dog	0.08 (IV)		2.8	31.5	58.2	(Salonen, 1989)
	Dog	0.08 (IM)		3.0	27.5	76.8	(Salonen, 1989)
	Cat	0.08 (IM)		3.5	29.5	81	(Salonen, 1989)
	Horse	0.007 (IV)	32.9 ± 7.21	2.2 ± 0.52	66.6 ± 9.9	51.3 ± 13.09	(Bettschart-Wolfensberger et al., 1999)
	Calf	0.04 (IV)	30.1 ± 4.0	1.75 ± 0.30	33.1 ± 5.5	44.4 ± 14.2	(Ranheim et al., 1998)
	Lactating dairy cows	0.04 (IV)	72.7 ± 30.7	1.21 ± 0.32	24.2 ± 6.5	52.7 ± 25.3	(Ranheim et al., 1999)
	Sheep	0.04 (IV)	37.2 ± 10	1.7 ± 0.26	44.2 ± 11.3	34.8 ± 7.3	(Ranheim et al., 2000)
	Sheep	0.03 (IM)	70 ± 17.4	3.9 ± 2.4	81.0 ± 21.5	32.7 ± 14.9	(Kastner et al., 2003)
Dexmedetomidine							
	Dog	0.02 (IV)		0.86 ± 0.22	20.1 ± 8.0	47 ± 14	(Kuusela et al., 2000)
	Young Ponies (aged)	0.0035 (IV)				19.8 (28.9)	(Bettschart-Wolfensberger et al., 2005)
Levomedetomidine							
	Dog	0.02 (IV)		2.68 ± 0.57	67.8 ± 11.5	38 ± 18	(Kuusela et al., 2000)
Clonidine							
	Sheep	0.006 (IV)		~ 5L		95	(Castro and Eisenach, 1989)

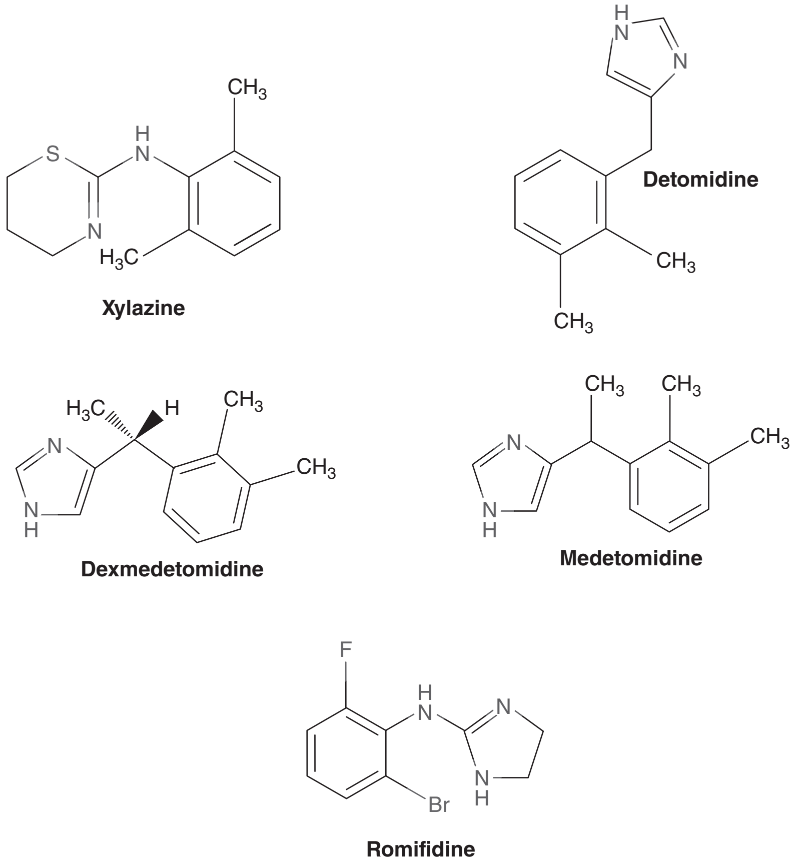


Figure 14.5 Alpha-2-agonists.

Systematic chemical name: N-(2,6-dimethylphenyl)-5,6-dihydro-4H-1,3-thiazin-2-amine (XX).

Xylazine was the first α_2-adrenergic agonists used in veterinary medicine and its clinical use was first described in the 1969 in a number of species (Clarke and Hall, 1969; Keller, 1969; Müller et al., 1969). Xylazine is often thought of as the prototypical α_2-adrenergic agonist; however, it does have one of the lowest $\alpha_2 : \alpha_1$ affinities (Table 14.2), and does not bind to imidazoline receptors. It is still the most commonly used α_2-agonist in horses and is also commonly used in lab animal and wildlife medicine. Interestingly, although rare, xylazine has been associated with aggressive behavior following administration in horses (Hubbell and Muir, 2004), which may be due to the excitatory effects of α_1-agonists in the CNS (see Section Central Nervous System Effects).

Cattle are particularly sensitive to the effects of xylazine and require a much reduced dose compared with other species and approximately one-tenth the dose compared with horses. The dose rate of other α_2-adrenergic agonists in cattle is similar to other species. Conversely, swine require two to three times the dose of xylazine compared to the dog, cat, or horse. These species differences are likely due to differences in G-protein signaling pathways (Törneke et al., 2003), as well as anatomical differences in α_2 and α_1-receptor distributions (Hellyer et al., 2003). Xylazine is the α_2-agonist most likely to cause vomiting in the dog, whereas almost all α_2-agonists cause vomiting in cats (Jovanovic-Micic et al., 1995; Sinclair, 2003).

Dosage

See Box 14.5.

Box 14.5 **Xylazine dosages**

Species	Dose and route
Dog and cat	0.1–1.0 mg/kg IV, IM
Cat emesis	0.4–0.5 mg/kg IV
Horse	0.02–1.0 mg/kg IV, IM
Bovine	0.01–0.1 mg/kg IV, IM
Ovine and caprine[a]	0.05–0.3 mg/kg IV, IM
Cervidae (deer)	0.5–4.0 mg/kg IM

[a] Xylazine can induce respiratory distress (see respiratory effects)

How supplied: Each ml of xylazine hydrochloride is equivalent to 20, 100, or 300 mg of base activity, methylparaben 0.9 mg, propylparaben 0.1 mg, sodium citrate dihydrate 5.0 mg and water for injection. pH adjusted with citric acid and sodium citrate.

Regulatory Information

Controlled drug status: Nonscheduled drug.

Withdrawal times:

Cattle: Milk withholding time is 24 hour; slaughter withdrawal time is 4 days.
Cervidae: Not approved for use during hunting season.

Veterinary Approved Products (Representative)

Xylazine is approved by the FDA for use in the dog, cat, horse, and deer.

Rompun® Injectable: xylazine HCl, 20 and 100 mg/ml (Bayer Healthcare, Animal Health Division)
AnaSed® Injectable: xylazine HCl, 20 and 100 mg/ml (Lloyd, Inc.)
Cervizine 300 Injectable: xylazine HCl, 300 mg/ml (Lloyd, Inc.)

Detomidine Hydrochloride

Synonym: Dormosedan.

Molecular formula: C12H14N2.HCl (Figure 14.5).

Molecular weight: 222.7 g/mol.

Systematic chemical name: 1H imidazole, 4-[(2,3-dimethylphenyl) methyl]-hydrochloride.

It is a white, crystalline, water-soluble substance. Detomidine is FDA approved only for use in the horse; however, it is used in bovines and to a lesser degree in small ruminants (Singh et al., 1989, 1997). It has a higher $\alpha_2 : \alpha_1$ ratio than xylazine (Table 14.2), and in horses, detomidine has the longest duration of action (Yamashita et al., 2000) and results in the longest duration of ataxia (England et al., 1992; Hamm et al., 1995). Although the pharmacodynamic effects of the various α_2-agonists at equipotent doses have been reported to be similar, except for the duration of action (Bueno et al., 1999; England et al., 1992), most clinicians believe that, in horses, detomidine provides more profound sedation and less weight shifting when sedated then other α_2-adrenergic agonists.

Detomidine was one of the first α_2-adrenergic agonists to be administered as a constant rate infusion in horses for standing surgical procedures (Daunt et al., 1993; Wilson et al., 2002). Horses sedated via constant rate infusion assume a base-wide stance and with appropriate local blocks will tolerate significant surgical manipulation.

Oral/transmucosal administration of injectable detomidine has been used with varying degrees of success in the number of species (Pollock and Ramsay, 2003; Ramsay et al., 2002; Sleeman and Gaynor, 2000; Grove and Ramsay, 2000). A combination of detomidine and ketamine administered orally in the cat proved more effective than a combination of ketamine and xylazine or medetomidine (Grove and Ramsay, 2000). Oral detomidine in the horse produced maximal sedation 30 minutes postadministration (Ramsay, 2002).

Detomidine Gel is a transmucosal product licensed for use in horses only. The bioavailability in horses is ~22% (Kaukinen et al., 2011), and effective sedation reached in the majority of horses 40 minutes after administration (Gardner et al., 2010). Detomidine gel has also been successfully used off label in dogs and ferrets (Hopfensperger et al., 2013; Phillips et al., 2015). The transmucosal gel, when applied to the oral mucosa of dogs in a small volume produces sedation or recumbency (depending on the dose) that has an onset of approximately 30–45 minutes, with a duration in dogs of approximately 90 minutes.

Dose

See Box 14.6.

Box 14.6 Detomidine dosages

Species	Dose and route	Reference
Horse	Parenteral: 5 – 40 μg/kg IV, IM CRI: Loading bolus 8.4 μg/kg IV 1st 15 min. 0.5 μg/kg/min IV 2nd 15 min 0.3 μg/kg/min IV 3rd 15 min 0.1 μg/kg/min IV Transmucosal Gel: 40 μg/kg between gum and cheek, or under tongue	
Cat	Oral: 500 μg/kg detomidine + 10 mg/kg ketamine	(Grove and Ramsay, 2000)
Dog	Transmucosal Gel: mild sedation 0.5 mg/m^2; higher sedation and lateral recumbency: 1.0 mg/m^2. (Average dose is 35 μg/kg.) A high dose of 2–4 mg/m^2 will produce more profound sedation. (Using the 7.6 mg/ml: Dormosedan Gel)	
Ferret	Transmucosal Gel: 2 mg/m^2 and 4 mg/m^2 (transmucosal) (Using the 7.6 mg/ml: Dormosedan Gel)	(Phillips et al., 2015)

How supplied: Each ml of detomidine has 10 mg/ml.

Veterinary Approved Products
Approved for use in horses

Liquid for injection, 10 mg/ml, Dormosedan (Zoetis, USA).
Gel for transmucosal application, 7.6 mg/ml, Dormosedan Gel (Zoetis, USA).

Regulatory information

Controlled drug status: Nonscheduled drug.

Withdrawal times: Not approved for use in horses intended for human consumption.

Extralabel use in cattle: 3 days meat, 72 hours milk.

Romifidine

Synonym: Sedivet.

Molecular formula: C9-H9-Br-F-N3 (Figure 14.5).

Molecular weight: 258.093.

Chemical name: 2-(2-Bromo-6-fluoroanilino)-2-imidazoline.

Romifidine is a more selective α_2-agonist than xylazine (Table 14.2) and in the horse produces less ataxia compared with equipotent doses of other α_2-adrenergic agonists (England et al., 1992). Romifidine improved the quality of recovery in horses when given preoperatively (Jaugstetter et al., 2002) or postoperatively (Woodhouse et al., 2013), which may reflect the longer duration of action of romifidine compared to xylazine or less ataxia, or both.

Romifidine causes similar cardiovascular depression in the cat (Selmi et al., 2004) and dog (Pypendop and Verstegen, 2001). There is a ceiling effect with respect to the cardiovascular changes induced by romifidine in the dog and horse (Pypendop and Verstegen, 2001; Freeman et al., 2002). A similar ceiling effect was reached for the level of sedation in the dog (<25–40 μg/kg IV) (Pypendop and Verstegen, 2001).

The amount of analgesia romifidine produces in the horse is controversial with some researchers finding no analgesic effects (Hamm et al., 1995), while others have documented analgesia (Moens et al., 2003; Spadavecchia et al., 2005). Neuraxial romifidine potentiates the effects of epidural morphine and lidocaine in cattle and goats (Fierheller et al., 2004; Kinjavdekar et al., 2003).

Doses
See Box 14.7. Romifidine is only approved for use in horses.

Box 14.7 Romifidine dosages

Species	Dose and route
Horse	40–120 μg/kg IV
Dog	1–40 μg/kg IV
Cat	5–200 μg/kg IM

Regulatory information

Controlled drug status: Nonscheduled drug.

Withdrawal times: Not approved for use in horses intended for human consumption.

Veterinary Approved Products
Romifidine is approved by the FDA for use in the horse.

Sedivet® 1% Injection, Romifidine HCl 10 mg/ml (Boehringer Ingelheim Vetmedica, Inc.).

Medetomidine and Dexmedetomidine
Medetomidine is a racemic mixture of two optical enantiomers, dexmedetomidine and levomedetomidine (1 : 1). The structures of these two enantiomers are mirror images of each other, but they each possess different biological activity. Both drugs have been or are marketed in the veterinary community.

Medetomidine

Synonym: Domitor.

Molecular weight: 200.283.

Molecular formula: C13-H16-N2 (Figure 14.5).

Chemical name: 4-[1-(2,3-dimethylphenyl)ethyl].

Medetomidine is a racemic mixture of two optical enantiomers, dexmedetomidine and levomedetomidine (1:1). The active component of medetomidine is dexmedetomidine; however, medetomidine does not appear to have simply half the potency of dexmedetomidine (Kuusela et al., 2000, 2001b). Levomedetomidine has minimal behavioral effect but likely contributes to the cardiovascular effects (Kuusela et al., 2000, 2001b).

Medetomidine produces profound reliable sedation and analgesia. Medetomidine is also used frequently to treat emergence delirium following anesthesia, particularly in patients with awakening from painful surgical procedures. Microdoses of 1–2 μg/kg are usually sufficient to quiet a patient and allow it to rest comfortably. Similarly, constant rate infusions of medetomidine are popular in the ICU setting to provide similar sedation, anxiolysis, and analgesia.

Dose

See Box 14.8.

Box 14.8 Medetomidine dosages

Species	Dose and route
Dogs	Single injection: 1–20 μg/kg IV, IM Constant rate infusion: 1–5 μg/kg/hour
Cats	Single injection: 1–40 μg/kg IV, IM Constant rate infusion: 1–5 μg/kg/hour
Horse	Single injection: 2–10 μg/kg IV, IM Constant rate infusion: 0.03–0.1 μg/kg/min

Veterinary Approved Products

Medetomidine is approved by the FDA for use in the dog.

Domitor®: Medetomidine HCl 1 mg/ml (Orion Corp.).

Regulatory Information

Controlled drug status: Nonscheduled drug.

Withdrawal times: Not approved for use in food-producing animals.

Dexmedetomidine

Synonyms: Dexdomitor, Precedex.

Molecular weight: 200.283.

Molecular formula: C13-H16-N2 (Figure 14.5).

Chemical name: 4-[(1R)-1-(2,3-dimethylphenyl)ethyl].

Dexmedetomidine is the dextro-enantiomer of the racemic drug, medetomidine. It was originally marketed for people (Precedex), but it is approved for use in dogs and cats in the USA. Since the dextro-enantiomer is the biological active form, it was assumed this form would need half the dose and produce less side effects than the racemic mixture of medetomidine. However, there appears to be a complex interaction between the dextro-enantiomer and levo-enantiomer to produce the pharmacodynamic effects of the racemic mixture of medetomidine (Kuusela et al., 2000, 2001b); therefore dosing is generally a little more than half the dose of medetomidine's expected effects. Clinically, at equipotent doses, sedation, analgesia, and cardiovascular effects are similar to medetomidine (Kuusela, 2004).

Representative Dosages

See Box 14.9.

Box 14.9 Dexmedetomidine dosages

Species	Dose and route
Dog	Single injection: 0.5–10 μg/kg IM, IV Constant rate infusion: 0.5–3.0 μg/kg/h IV
Cat	Single injection: 1–20 μg/kg IM, IV Constant rate infusion: 0.5–3.0 μg/kg/h IV

Veterinary Approved Products

Dexmedetomidine is approved by the FDA for use in the dogs and cats.

Dexdomitor®: Dexmedetomidine HCl, 0.1 mg/ml and 0.5 mg/ml (Zoetis).
Human formulation is Precedex®, 4 or 100 μg/ml.

Regulatory Information

Controlled drug status: Nonscheduled drug.

Withdrawal times: Not approved for use in food-producing animals.

Species Differences

There are species differences to the sedative effects of α_2-adrenergic agonists. In general, from the most sensitive to least sensitive are: bovine > camelids > small ruminants > cat, dog, and horse > porcine > small laboratory animals.

Cattle: Ruminants generally have an exaggerated response to the sedative effects of α_2-agonists and, in particular, xylazine. It has been suggested that cattle may have some central α_1-receptors that contribute to sedation, or this may be due to them having an α_{2D}-adrenergic receptors (Schwartz and Clark, 1998). In particular, cattle need ~1/10 the dose of xylazine compared with horses.

Sheep: Xylazine causes the activation of pulmonary intravascular macrophages (PIM), which results in acute lung injury (see Section Respiratory Effects).

Horse: Equipotent intravenous doses for sedation in the horse are considered to be the following: xylazine (1 mg/kg), medetomidine (5–10 μg/kg), romifidine (40–80 μg/kg), and detomidine (20–40 μg/kg) (England et al., 1992; Yamashita et al., 2002; Bryant et al., 1991).

Adverse effects/ contraindications

Alpha-2-adrenergic agonists should not be used in animals with compromised cardiac output, such as preexisting heart disease, especially those with bradydysrhythmia, poor myocardial contractility, obstructive valvular disease, dehydration, hypovolemia, or sepsis.

It is possible to see sudden arousal from α_2-adrenergic agonist-induced sedation that may proceed to aggression. This may be due to sensitivity to sound and or touch as well as the excitatory effects of α_1-receptors in the CNS (Clarke and Hall, 1969; Hubbell and Muir, 2004).

Caution should also be used in patients with diabetes mellitus (see Section Glucose).

Drug Interactions

Alpha-2-adrenergic agonists potentiate the CNS depressant effects of other sedative agents such as propofol, ketamine, or opioids. The dosage of induction agents is greatly reduced and there is a profound MAC-sparing effect. The respiratory depression of α_2-adrenergic agonists is potentiated by other respiratory depressant, especially opioids. This combination is more likely to cause hypercapnia and hypoxemia compared to α_2-adrenergic agonists alone.

Overdose/Acute Toxicity

Treatment for the overdose of an α_2-adrenergic agonist is centered on the use of α_2-adrenergic antagonists titrated to effect (e.g., atipamezole or yohimbine, see Sections Atipamezole and Yohimbine). Careful monitoring of the cardiovascular and respiratory systems is required. Other supportive therapy may include external regulation of body temperature and monitoring of blood glucose levels.

Alpha-2-Adrenergic Antagonists

History/ Introduction

Alpha-2-adrenergic receptor antagonists are used primarily in veterinary medicine to reverse the wanted or unwanted effects of the α_2-adrenergic agonists. The ability to reverse the α_2-adrenergic agonists increases their appeal for use in general veterinary medicine as well as their use for the chemical restraint for zoo animals and exotic wildlife. Three antagonists are available for use in veterinary medicine: atipamezole, yohimbine, and tolazoline.

Classification

Drugs in this class are all α_2-adrenergic receptor ligands that are competitive antagonist at the α_2-adrenergic receptor site. They all have varying degrees of antagonism at the α_1-adrenergic receptor site as well.

Mechanism of Action

Alpha-2-adrenergic antagonist drugs act by competitively binding to the α_2-adrenergic receptor and preventing activation. Like α_2-adrenergic receptor agonists, antagonists vary by their selectivity of binding α_2 or α_1-adrenergic receptors. Binding without activation reverses and/or attenuates the sedation, anxiolysis, analgesia, and the central and peripheral cardiovascular changes induced by α_2-adrenergic agonists; however, behavioral and cardiovascular effects vary by species and drug (Carroll et al., 2005; Hubbell and Muir, 2006).

Indications

Alpha-2-receptor antagonists are routinely used to reverse the sedative or physiological (cardiovascular or respiratory) effects of α_2-agonist drugs. It is logical and generally recommended to choose an antagonist with a similar specificity as the agonist given. For example, xylazine is commonly reversed with yohimbine and dexmedetomidine is reversed with atipamezole; however, reversal can and does occur with more or less specific reversal agents. Some species differences do exist (see Section Specific Drugs).

Dosages for full reversals are given below. However, many clinicians routinely give antagonists at lower doses since some of the α_2-agonist has already been metabolized and leaving some sedative and analgesic effects results in a less excited return to function.

Reversal of Analgesia

Abrupt and compete reversal of α_2-adrenergic agonist drugs may lead to a sudden increase in sympathetic activity and nociception (reversal of analgesia) that at a minimum may be unpleasant and, at worst, inhumane. In various animal studies, the use of atipamezole to reverse the effects of α_2-adrenergic agonists increased the nociceptive response by blocking the norepinephrine feedback inhibition of pain sensation (Pertovaara et al., 2005). Following α_2-receptor antagonism, patients need to be assessed for pain levels and may be administered other analgesic such as opioids or nonsteroidal antiinflammatory drugs (NSAIDs).

Specific drugs

Atipamezole

Synonyms: Antisedan, Atipamezol, Atipamezolum.

Molecular weight: 212.294.

Molecular formula: C14-H16-N2 (Figure 14.6).

Chemical name: 4-(2-ethyl-1,3-dihydroinden-2-yl)-3H-imidazole.

Atipamezole is a highly specific α_2-receptor antagonist that was designed to reverse the effects of medetomidine and dexmedetomidine. It is approved only for intramuscular use in dogs, but is commonly used off label in cats, horses, and exotic species. Along with its high specificity for the α_2-receptor, atipamezole does not bind at β-adrenergic, 5-HT, histaminergic, muscarinic, or dopaminergic receptors, which decreases side effects with administration(Tranquilli et al., 2007).

Atipamezole acts rapidly, producing shorter reversal time for lambs sedated with medetomidine 30 μg/kg IV as compared to yohimbine (Ko and McGrath, 1995), allowing the lambs to walk within 2–3 minutes as compare 15 minutes. However, there are various reports of atipamezole not providing full reversal in horses administered the less specific drug detomidine (Zeiler, 2015), therefore some animals require repeated dosing to get a full reversal and some animal can become resedated later.

Aside from their use as reversals, α_2-antagonists have been used as therapeutics. Atipamezole has also been used to reverse the toxic effects of amitraz poisoning in the dog (Hugnet et al., 1996) and cat (Andrade et al., 2006). Since I_2-imidazoline receptors are involved in the pathogenesis of Parkinson's disease (Gentili et al., 2006), there are ongoing efforts investigating the uses of atipamezole in cognitive enhancement functions (nootropic), brain injury, and as a potential antiparkinsonian therapy (Pertovaara et al., 2005). Yohimbine is used to treat erectile dysfunction in men, but there is no record of this use in veterinary medicine.

Representative Dosage

Atipamezole is approved for IM use in dogs only (Box 14.10). The product label for atipamezole lists the dosage in μg/m^2 (see below). However, many veterinarians administer atipamezole at an equal volume (ml) to the amount of medetomidine/ dexmedetomidine given (1.0 mg/ml and 0.5 mg/ml, respectively). Given each drug's concentration, this is equivalent to reversing with five times the concentration of medetomidine/ dexmedetomidine.

Box 14.10 Atipamezole dosages

Species	Dose and route
Dogs	Reversal of α_2-agonist: 3750 μg/m^2 IM (product label dosage)
	Reversal of α_2-agonist: 0.1–0.3 mg/kg IM
Cats	Reversal of α_2-agonist: 0.1–0.3 mg/kg IM
Horses	Reversal of α_2-agonist: 0.03–0.06 μg/kg IV, IM

Veterinary Approved Products

Atipamezole HCL: Antisedan®, 5 mg/ml (Zoetis).

Regulatory Information

Controlled drug status: Nonscheduled drug.

Withdrawal times: Not approved for use in food-producing animals.

Yohimbine

Synonyms: Yobine, Antagonil, Yohimbin.

Molecular formula: C21-H26-N2-O3 (Figure 14.6).

Chemical name: Yohimban-16-alpha-carboxylic acid, 17-alpha-hydroxy-, methyl ester.

Yohimbine is an indole alkaloid that is derived from the bark of the *Pausinystalia yohimbe* tree or the root of the *Rauwolfia serpentina* plant. It is a nonselective α_2-receptor antagonist and at high doses can activate α_1-adrenoreceptors, dopamine and serotonin receptors, and inhibit monoamine oxidase production (DiMaio Knych et al., 2011). It is approved for use in dogs and cervidae, but is commonly used to reverse the effects of the α_2-agonist xylazine in dogs, cats, horse, cattle, and exotic species. Pharmacokinetic data for various species are summarized in Table 14.4. Rapid IV administration can result in hypotension from vasodilation as well as CNS excitement. Clinicians will often administer a fraction (25–25%) of the dose IV and the remainder IM, to help prevented unwanted side effects.

Yohimbine is classified as a Class 2 foreign substance by the Association of Racing Commissioners International (ARCI) (DiMaio Knych et al., 2011).

Yohimbine has been used in human medicine to treat erectile dysfunction (Riley, 1993).

Representative Dosages

See Box 14.11.

Box 14.11 Yohimbine dosages

Species	Dose and route
Dogs and cats	Reversal of α_2-agonist: 0.11 mg/kg IV; 0.25–0.5 mg/kg IM
Horses	Reversal of α_2-agonist: 0.02–0.075 mg/kg IV
Cattle and sheep	Reversal of α_2-agonist: 0.125–0.2 mg/kg IV

Veterinary Approved Products

Yohimbine is approved by the FDA for use in the dog and cervidae (elk and deer).

Yobine® Injectable Solution: Yohimbine HCl, 2.0 mg/ml (Akorn, Inc.).
Antagonil®: Yohimbine, HCl 5.0 mg/ml (Wildlife Laboratories, Inc.).

Regulatory Information

Controlled Drug Status: Nonscheduled drug.

Withdrawal times: Not approved for use in food-producing animals. Should not be used 30 days prior to hunting season.

Tolazoline

Synonym: Tolazine.

Molecular weight: 160.2188.

Molecular formula: C10-H12-N2 (Figure 14.6).

Chemical name: 2-benzyl-4,5-dihydro-1H-imidazole.

Tolazoline is a synthetic imidazoline derivative that functions as a nonselective α-adrenergic receptor antagonist. Tolazoline is the only FDA-approved drug for the reversal of xylazine-induced sedation in the horse, but is commonly used to reverse the effects of xylazine in cattle, camelids, and exotic species. In addition to antagonism of the α_2-receptor, tolazoline has histaminergic and cholinergic effects (Tranquilli et al., 2007) as well as direct effects on the vascular endothelium, which decreases systemic vascular resistance (vasodilation) and increasing venous capacitance (Short, 1987; Tranquilli and Thurmon, 1984). As with all the antagonists, tolazoline should be administered slowly and to effect to prevent unwanted side effects.

Tolazoline is generally thought to provide better reversal for cattle and camelid species (Hsu et al., 1987, 1989); however, there have been reports of adverse effects and fatalities in these species with appropriate and inappropriate dosing (Read et al., 2000). Tolazoline reverses the effects of detomidine in the horse better than atipamezole (Hubbell and Muir, 2006).

At doses up to five times recommended, tolazoline caused signs of gastrointestinal hypermotility, transient diarrhea, and colic (Lloyd Laboratories, 1996).

While approved for IV use, tolazoline was shown to effective when administration via the endotracheal route (Paret et al., 1999a).

The pharmacokinetics for antagonists in several species are summarized in Table 14.4.

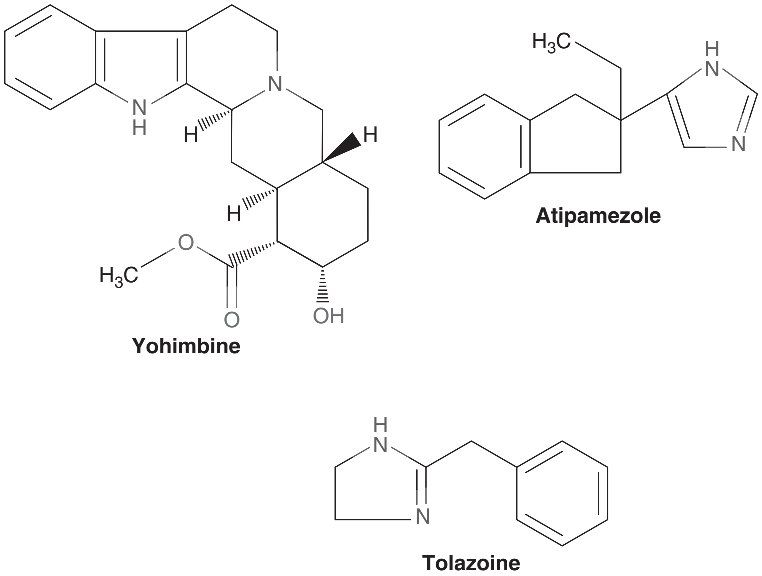


Figure 14.6 Alpha-2 antagonists.

Table 14.4 Alpha-2-adrenergic antagonists pharmacokinetics

Drug	Species	Dose	Mean residence time (minutes)	Volume of distribution (l/kg)	Clearance$_{Tot}$ (ml/kg/min)	Elimination half-life (min)	Reference
Yohimbine	Steers	0.25 mg/kg IV	86.7 ± 46.2	4.9 ± 1.4	69.6 ± 35.1	46.7 ± 24.4	(Jernigan et al., 1988)
	Horse	0.075–0.15 mg/kg IV	106 ± 72.1 to 118.7± 35.0	2.7 ± 1.0 to 4.6 ± 1.9	34.0 ± 19.4 to 39.6 ± 16.6	52.8 ± 27.8 76.1 ± 23.1	(Jernigan et al., 1988)
	Dog	0.4 mg/kg IV	163.6 ± 49.7	4.5 ± 1.8	29.6 ± 14.7	104.1 ± 32.1	(Jernigan et al., 1988)
Tolazoline	Dog	6 mg/kg via ET tube[a]		1.65 ± 0.3	10.9 ± 4.8	156 ± 81	(Paret et al., 1999b)
	Horse	4 mg/kg		1.68 ± .38	13.7 ± 3.0	160 ± 60	(Casbeer and Knych, 2013)
	Lambs	2 mg/kg IV		2.5 ± 0.7	27.1 ± 7.6[b]		(Ward et al., 1984)
Atipamezole							
	Dog						
	Cat						
	Horse						
	Dairy cow	0.2 mg/kg IV	38.3 ± 15.7	1.77 ± 0.64	48.1 ± 13.1	35.2 ± 17.9	(Ranheim et al., 1999)[c]
	Calf	0.2 mg/kg IV	48.1 ± 9.2	2.97 ± 1.4	42.9 ± 3.9	52.1 ± 7.0	(Ranheim et al., 1998)[c]
	Sheep	0.2 mg/kg IV	34.4 ± 7.9	2.0 ± 0.54	56.3 ± 9.5	34.2 ± 11.9	(Ranheim et al., 2000)[c]

[a] Following the use of xylazine and thiopental.
[b] This was in lambs 17 days of age. Clearance increased to 60 ml/kg/min by the age of 28 days.
[c] This was following medetomidine 0.04 mg/kg IV.

Representative Dosages

See Box 14.12.

Box 14.12 Tolazoline dosages

Species	Dose and route
Dogs and cats	Reversal of α_2-agonist: 4 mg/kg IV slowly
Horses	Reversal of α_2-agonist: 4 mg/kg IV slowly
Cattle	Reversal of α_2-agonist: 2–4 mg/kg IV slowly (calves 1 mg/kg IV slowly)
Llamas	Reversal of α_2-agonist: 2 mg/kg IM

Veterinary Approved Products

Tolazoline is approved by the FDA for use in the horse.

Tolazine^{TM} Injection: tolazoline HCl, 100 mg/ml (Lloyd, Inc.).

Tolazoline HCL: tolazaoline HCL, 200 mg/ml (ZooPharm).

Regulatory Information

Controlled drug status: Nonscheduled drug.

Withdrawal times: Not approved for use in horses intended for human consumption.

Pharmacodynamic Effects

Central nervous system effects: The use of α_2-adrenergic antagonists will reliably reverse the sedation, ataxia, and physiological effects caused by the α_2-adrenergic agonist in a dose-dependent manner. This is due to the blockade of the norepinephrine feedback inhibition mediated by the presynaptic α_2-adrenergic receptor activation.

All three antagonists have the potential to cause CNS excitement (agitation, fasciculations, tremors, and hyperesthesia). Yohimbine has the potential to cause seizures at higher doses via GABAergic and NMDA-mediated pathways (Dunn and Corbett, 1992). Atipamezole has only slight effects on behavior under familiar conditions, but increases neophobia (Haapalinna et al., 1997). Additionally, reversal of α_2-agonist drugs also reverses analgesia (not just sedation), which can contribute to an excited, painful recovery. It is always recommended that α_2-receptor antagonists be administered slowly and to effect to minimize these adverse effects.

Cardiovascular effects: In general, α_2-antagonists reverse or attenuate the cardiovascular changes caused by α_2-agonist drugs (e.g., xylazine) such as increased systemic vascular resistance and bradycardia. The actual cardiovascular responses are difficult to predict and are dependent on the initial agonist used, the timing of the reversal (e.g., if during the initial phase of intense peripheral vasoconstriction), the route of administration of the antagonist, and the species in question (Zeiler, 2015; Kolliasbaker et al., 1993; Savola, 1989).

Additionally, some of the α_2-antagonists are cardioprotective, both alone and when attenuating the results of α_2-agonists. Yohimbine caused an increase reperfusion of the left ventricle while also causing a reduction in left ventricular end diastolic pressure and release of lactate dehydrogenase (Sargent et al., 1994). Xylazine has been shown, using in vitro techniques, to cause coronary vasoconstriction in the dog, which was reversed by atipamezole (Teng and Muir, 2004).

Respiratory effects: Alpha-2-antagonists can reverse or attenuate the respiratory depression of α_2-agonists. At routine doses in healthy animals, α_2-agonists cause minimal changes to ventilation so antagonism of those effects do not change CO_2 or O_2 levels (Kolliasbaker et al., 1993). However, for patients with exaggerated clinical effects due to high doses, comorbidities, or coadministration of other CNS depressants, antagonism should reverse respiratory depression.

Tachypnea and hypoxemia can be seen secondary to α_2-agonist use in sheep due to activation of pulmonary intravascular macrophages (PIM) and production of acute lung injury and pulmonary edema. α_2-adrenergic antagonists attenuate the tachypnea induced by medetomidine in lambs and ewes; however, these agents failed to correct the reduction in arterial oxygen partial pressure, likely due the acute lung injury sustained (Ko and McGrath, 1995; Hsu et al., 1989; Celly et al., 1997).

Tolazoline has been used to treat pulmonary hypertension in newborns and has been shown to reduce pulmonary vascular resistance and counteract the hypoxic pulmonary vasoconstriction reflex (Paret et al., 1999b).

Muscular effects: All the α_2-adrenergic antagonists have been shown to reverse or attenuate the ataxia and muscle weakness induced by the α_2-adrenergic agonists (Hsu et al., 1989; Hubbell and Muir, 2006).

Adverse Effects/ Contraindications

Sudden death has been anecdotally reported in patients given α_2-receptor antagonists IV, presumptively from cardiovascular collapse. One possible reason for acute cardiovascular collapse could be the preservative, methylparaben, in atipamezole, which can cause histamine release and therefore reduce blood pressure. However, it is more likely that when the antagonist is given in large doses IV to patients with peripheral vasoconstriction, reflex bradycardia, and high vagal tone, the rapid relaxation of vessel tone coupled with bradycardia induces the cardiovascular collapse. Caution should be used when quickly administering an antagonist while a patient is in the acute vasoconstrictive phase. Intramuscular administration is less likely to produce acute cardiovascular changes.

CNS excitement and delirium leading to seizure activity have been reported, particularly at high doses (Ranheim et al., 2000; Read et al., 2000). Clinicians must investigate if the excitement is due to reemergence and perhaps pain or due to central CNS excitement. Many clinicians will only administer partial doses (25–50%) to minimize these effects.

The safety of α_2-adrenergic antagonists has not been established in pregnant or lactating animals, animals intended for breeding purposes, or metabolically unstable patients.

Species Differences

Cattle and small ruminants sedated with xylazine appear to have better reversal when treated with tolazoline compared with yohimbine (Hikasa et al., 1988). This is likely due to differing receptor types and binding affinities of the agonist as well as the antagonist (see Section Yohimbine).

Llamas appear to be more sensitive to the adverse effects of tolazoline compared to other species. It has been recommended to administered tolazoline at 2 mg/kg intramuscularly to minimize these adverse effects (DuBois et al., 2004; Read et al., 2000).

Tolazoline in captive bears has proven to be ineffective for the reversal of α_2-adrenergic agonists and is therefore not recommended in this group of animals (Cattet et al., 2003).

Overdose/Acute Toxicity

Tolazoline: Gastrointestinal disturbances, agitation, muscle fasciculations, tachycardia, mild hypertension, and, at high doses, ventricular arrhythmias and death were seen with the administration of tolazoline alone. Overdoses of tolazoline at five times the recommended dose have been associated with fatalities in horses (Lloyd Laboratories, 1996).

Atipamezole: Healthy dogs have tolerated up to ten times the recommended dose of atipamezole (Zoetis, 2015). No significant clinical signs were seen following a regular dose of atipamezole in nonsedated dogs (Zoetis, 2015). Signs of an overdose included excitement, panting, trembling, vomiting, mild diarrhea, and scleral injection.

Drug Interactions

Drug interaction with α_2-antagonists generally are either due to an increase in chance for hypertension (due to increase in norepinephrine) or due to exaggerated CNS stimulation (Trevor and Way, 2001). Therefore these drugs should be used cautiously with any patient that is already hypertensive or being administered drugs that might enhance heart rate or blood pressure (e.g., positive inotropes or anticholinergics). Similarly, these drugs should be given with caution with other CNS stimulants (e.g., caffeine).

Guaifenesin

History

Guaifenesin has a long history as a medical therapeutic both as a muscle relaxant as well as expectorant. It has been used as an adjunct to anesthesia in the horse since 1949 and has been used in the USA since 1965. It is still commonly used as an expectorant in people, as it is combined with dextromethorphan or used alone in many cough and cold remedies sold over the counter but, more commonly in veterinary medicine, is it administered primarily to horses for its sedation and muscle relaxation properties.

Classification

Chemistry

Synonyms: GG, glyceryl guaiacolate.

Molecular formula: C10-H14-O4 (Figure 14.7).

Molecular weight: 198.2166.

Chemical name: 3-(0-methoxy-phenoxy)-1,2-propanediol.

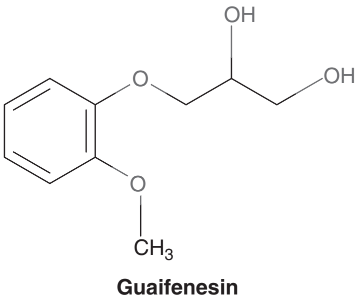


Figure 14.7 Guaifenesin.

Mechanism of Action

Guaifenesin is a central-acting skeletal muscle relaxant, but its exact mechanism of action is unknown. It selectively depresses or blocks nerve impulse transmission at the internuncial neuron level of the spinal cord, brainstem, and subcortical areas of the brain causing sedation and muscle relaxation (Muir and Hubbell, 2014; Haga et al., 2000).

Indications

In veterinary medicine guaifenesin is used as an adjunct anesthetic primarily in equine and ruminant patients, both during induction and in part of total intravenous anesthesia combinations. It is used to smooth induction and recovery as well as for decreasing the amount of induction drug needed (Brouwer, 1985). When used alone, animals may become recumbent, but surgical anesthesia is not achieved.

Physiological Effects

CNS effects: Guaifenesin is a central-acting skeletal muscle relaxant that also has CNS depressant effects. It selectively depresses or blocks nerve impulse transmission at the internuncial neuron level of the spinal cord, brainstem, and subcortical areas of the brain, causing sedation and muscle relaxation (Muir and Hubbell, 2014). Although the mechanism of action is unknown it produces CNS depression (not just muscle relaxation) based on behavior and EEG analysis (Haga et al., 2000).

Cardiovascular system: In the horse, initially there is a transient decrease in arterial blood pressure, which returns to normal, but myocardial contractile force and heart rate are relatively preserved (Hubbell et al., 1980). Many studies, in many species, have looked at the effects of guaifenesin on cardiac function when used in conjunction with other anesthetics such as ketamine, thiopental, and xylazine (Benson et al., 1985; McMurphy et al., 2002; Picavet et al., 2004). Thus, it is difficult to interpret the cardiovascular effects with respect to guaifenesin alone. However, it is likely that the additional CNS depression and muscle relaxation of guaifenesin allows for smaller doses of more-cardiovascular-depressing drugs such as thiopental or xylazine to be used. Thus guaifenesin may be useful in preserving cardiovascular function when used with other anesthetic drugs.

Respiratory: Even though guaifenesin is a skeletal muscle relaxant, in therapeutic amounts, it does not cause paralysis of the respiratory muscles (intercostal and diaphragm). Often, respiratory rate is increased but minute ventilation is unchanged (Hubbell and Muir, 2006; Muir and Hubbell, 2014; Tranquilli et al., 2007). At doses greater than needed to obtain recumbency, respiratory depression might become significant.

Muscular effects: Guaifenesin produces generalized skeletal muscle relaxation. At recommended doses, it does not affect respiratory muscles enough to significantly alter ventilation. The laryngeal and pharyngeal muscles are relaxed, which facilitates intubation of the trachea (Muir and Hubbell, 2014). At excessive doses, guaifenesin can produce a paradoxical increase in muscle rigidity (Muir and Hubbell, 2014).

Miscellaneous: Guaifenesin crosses the placenta and can be detected in the plasma of the fetus (Hubbell et al., 1980). Adverse effects of guaifenesin in newborns have not been reported.

Analgesia: Previous editions of this book have suggested that guaifenesin is analgesic, but there is little evidence to support that claim.

Pharmacokinetics

When administered to horses as a single agent, recumbency occurs in ~2 minutes and can last ~6 minutes. Sedation from a single dose may last 15–30 minutes. There is gender difference in the half-life of guaifenesin in ponies: males have a $T_{1/2}$ of ~85 minutes whereas in the female $T_{1/2}$ is ~60 minutes (Davis and Wolff, 1970).

Metabolism

Guaifenesin undergoes biotransformation in the liver to a glucuronide, which is then excreted in the urine (Davis and Wolff, 1970).

Adverse Effects/ Contraindications

The manufacturer cautions against administration with physostigmine, but does not explain the interaction. The use of guaifenesin in cows at concentrations exceeding 5% has been associated with red blood cell hemolysis (Wall and Muir, 1990).

Dose

Guaifenesin has a moderately high therapeutic index as three to four times the dose required to produce recumbency of the horse can be administered before death occurs (Funk, 1973). The primary disadvantage in the use of guaifenesin is the large volume of solution required to produce relaxation.

For horses the dose indicated on the label is 110 mg/kg IV. For preanesthetic sedation (prior to administration of other IV anesthetics) the dose is 75–110 mg/kg IV (lower doses used when used in combination with other CNS depressants such as thiopental or ketamine). For cattle the dose is 50–100 mg/kg IV.

Species Differences

Horse: Horses are still routinely sedated and anesthetized using guaifenesin. Generally, it is combined with or given immediately prior to other anesthetic drugs, such as xylazine, ketamine, or thiopental (see Section Triple Drip). Guaifenesin aids in producing a smooth induction and recovery as well as minimizing the amount of other drug needed to produce the desired effect. The administration of xylazine (1.1 mg/kg) prior to guaifenesin reduces the IV dose necessary to induce lateral recumbency in adult horses (Hubbell et al., 1980). When using guaifenesin for sedation immediately prior to induction with ketamine or thiopental, it should be administered until the horse begins to knuckle the fore or hind limbs. At that point, the guaifenesin should be discontinued and the induction agent immediately given. Hemolysis of red blood cells (as seen in cattle) has not been associated with guaifenesin concentrations up to 10% in horses (Grandy and McDonell, 1980).

Cattle: The use of guaifenesin in cattle and small ruminants is similar to that of horses. The use of guaifenesin in concentrations exceeding 5% has been associated with a dose-dependent red blood cell hemolysis (Wall and Muir, 1990).

Dogs: Guaifenesin had been used to treat strychnine poisoning in dogs (Bailey and Szabuniewicz, 1975), but is rarely used for sedation or anesthesia of dogs.

Triple Drip

Guaifenesin has been extensively used in combination with ketamine and xylazine. In horses, the mixture is commonly comprised of guaifenesin (50 mg/ml), ketamine (0.5–2 mg/ml), and xylazine (0.5–1.0 mg/ml). In cattle and small ruminants, guaifenesin (50 mg/ml) has been combined with ketamine (1–2 mg/ml) and xylazine (0.05–0.1 mg/ml). These combinations can be administered as induction agents, but are more routinely administered following anesthesia induction for maintenance of anesthesia. They should be administered to effect, but generally are administered ~1–2 ml/kg/h (Tranquilli et al., 2007). Guaifenesin has similarly been combined with detomidine (40 μg/ml) and ketamine (4 mg/ml), and administered at a continuous infusion rate of 0.8 ml/kg/min (Taylor et al., 1998).

Guaifenesin (50 mg/ml) can also been mixed with thiopental (2 mg/ml), and similarly used for maintenance of anesthesia at ~1.5 ml/kg/h (Muir and Hubbell, 2014).

Drug Interactions

Guaifenesin is often mixed with ketamine and xylazine ("triple drip") and with barbiturates with apparent stability. The manufacturer cautions against administration with physostigmine, but does not explain the interaction.

Overdosage/ Acute Toxicity

Guaifenesin has a wide therapeutic margin. Overdose can be associated with respiratory depression and muscle rigidity. Elimination in horses is rapid. Therefore, an overdose can be managed with supportive care until plasma levels decrease.

The use of guaifenesin in cows at concentrations exceeding 5% has been associated with red blood cell hemolysis (Wall and Muir, 1990).

Regulatory Information

Guaifenesin is FDA approved as a skeletal muscle relaxant for use in horses, but not for use in meat-production animals. Guaifenesin is approved for use in people as an expectorant and included in over-the-counter medications alone, or combined with dextromethorphan in cough and cold remedies.

Controlled drug status: Guaifenesin is not a scheduled controlled drug.

Withdrawal time: Guaifenesin is not approved for food-producing animals.

Extra-label use in food animals: 3 days for meat and 48 hours for milk.

Veterinary Products

Guailaxin: sterile powder 50 g (Fort Dodge Animal Health).

Gecolate/Glycodex Injection: 5% solution (50 mg/ml) (Summit Hill Laboratories).

How Supplied

Guaifenesin is supplied as a 5% (50 mg/ml) solution or as a white powder that is reconstituted to a 5% solution with water or 5% dextrose solution. It is not readily water soluble and easily precipitates out of solution at 22°C (72°F) or cooler (Funk, 1973). Warming and agitation usually eliminate the precipitate.

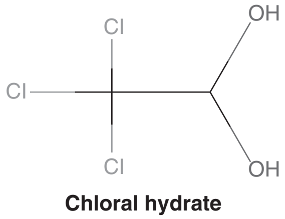


Figure 14.8 Chloral hydrate.

Chloral Hydrate

History

Chloral hydrate is the ingredient in the notorious “Mickey Finn,” named after a Chicago bartender who used the sedative properties combined with alcohol to rob his victims. It was introduced into medicine as a hypnotic in 1869 (Gauillard et al., 2001) and was among the first CNS depressants to be used in veterinary medicine. Chloral hydrate is rarely used to treat insomnia in people and for sedation in children, but is no longer used in conjunction with human anesthesia. It is a mutagen and a potential carcinogen and is rarely if ever used in veterinary medicine. This drug is discussed in greater detail in the seventh edition of this textbook.

Classification

Chemistry

Molecular formula: C2-H3-Cl3-O2 (Figure 14.8).

Molecular weight: 165.403.

Chemical name: 1,1,1-Trichloro-2,2-dihydroxyethane.

Chloral hydrate is the end product of chlorination of acetic aldehyde and addition of water. Chloral hydrate volatilizes on exposure to air and has an aromatic, penetrating odor. One gram of chloral hydrate is soluble in 0.25 ml water and in 1–2 ml of fat solvents.

Mechanism of Action

The mechanism of action of chloral hydrate is unknown, but it has been hypothesized to be a GABA agonist based on its synergy of action with other GABA agonists (ethanol, benzodiazepines) and the fact that the benzodiazepine antagonist, flumazenil, has been successfully used to treat chloral hydrate overdose in people (Gauillard et al., 2001).

Indications

Chloral hydrate has been used for general sedation and as an adjunct to anesthesia primarily in horses and cattle. It is rarely used in modern veterinary practice.

Physiological Effects

Chloral hydrate is a sedative hypnotic drug. It produces a dose-dependent sedation with a wide margin of safety when used at sedative doses, and was historically used as a sleep adjunct in people. It is, however, a poor anesthetic and when administered at anesthetic doses, the margin of safety is narrow. At anesthetic doses (compared with sedative doses), chloral hydrate produces significant respiratory and cardiovascular depression. Although chloral hydrate produces CNS depression, it does not provide analgesia and muscle relaxation is poor.

When administered IV, the sedative effects are seen almost immediately. Following oral administration, sedation occurs in 30–60 minutes. Choral hydrate is metabolized by alcohol dehydrogenase into trichloroethanol (TCE) and trichloroacetic acid. TCE is an active metabolite (Gauillard et al., 2001). The half-life of chloral hydrate is short (minutes), but the half-life of TCE is greater than 8 hours in humans (Gauillard et al., 2001).

The effects of chloral hydrate can be intensified when administered with other CNS depressants. Interestingly, chloral hydrate slows ethanol metabolism, thereby enhancing the effects of ethanol.

Chloral hydrate is irritating to the esophagus and GI tract when administered orally and should be administered in a diluted solution or following a meal. When administered intravenously, chloral hydrate can cause tissue necrosis and sloughing when administered perivascularly. When administered intraperitoneally, pain, ileus, and fibrosis may occur.

Dose

Cattle: The dose used for sedation is 0.025–0.05 ml/kg IV. To produce general anesthesia, the dose is 0.1–0.25 ml/kg IV until the desired effect is produced. Cattle usually require a lower dosage on the basis of body weight compared with horses. Oral administration is not recommended due to GI irritation.

Horses: The dose for sedation is 0.025–0.05 ml/kg IV. To produce general anesthesia, the dose is 0.1–0.25 ml/kg IV until the desired effect is produced. Oral administration is not recommended due to GI irritation.

Regulatory Information

Chloropent (chloral hydrate, magnesium, and pentobarbital) is approved for use in cattle and horses.

Controlled drug status: Chloral hydrate is Schedule IV controlled drug.

Withdrawal times: Chloropent (chloral hydrate, magnesium, and pentobarbital) is approved for use in dairy and beef cattle. FARAD lists a zero meat withdrawal time, but no recommendations for milk following sedation with chloropent.

Not intended for use in horses intended for human consumption.

Veterinary Products

Chloropent: Zoetis, approved for use in horse and cattle.

How Supplied

Presently, chloral hydrate is approved only for use in combination with pentobarbital and magnesium sulfate (chloropent) for anesthesia of cattle and horses. Each ml contains 42.5 mg of chloral hydrate, 8.86 mg of pentobarbital, and 21.2 mg of magnesium sulfate in 33.8% propylene glycol and 14.25% ethyl alcohol.

The addition of magnesium (mixture of 12% chloral hydrate and 6% magnesium sulfate) to a chloral hydrate solution increases the muscle relaxation and duration of action of chloral hydrate. The combination of chloral hydrate, magnesium sulfate, and pentobarbital sodium (previously marketed as Chloropent and Equithesin) provides some of the desirable depressant actions of each compound but minimizes the adverse effects of each by using smaller doses of each individual drug.

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15

Local Anesthetics

Thomas W. Vickroy

Introduction

Agents that are classified as local anesthetics act reversibly to prevent transmission of electrical impulses in nerve fibers and other excitable tissues. In most common applications, local anesthetics are administered to animals with the intent to exert actions that are restricted to regions of the body proximal to the site(s) of drug administration. However, as discussed elsewhere in this text, some agents are administered in different ways with the intent to produce broader systemic actions and, in that context, their actions are not classified as local anesthesia. The most widely accepted mechanism by which these drugs are thought to act involves direct binding to, and reversible blockade of, voltage-gated sodium ion (Na^+) channels in cell membranes. This singular action prevents Na^+ ion entry into nerve cells during periods of intense membrane depolarization, which prevents transmission of nerve impulses. The primary consequence of local anesthetic action is a reversible graded attenuation of sensation to pain and other sensory stimuli within affected body regions. The ability of local anesthetics to produce a selective decrease in pain sensitivity is noteworthy insofar as those effects occur without significant alteration in an animal's level of consciousness nor any substantial depression of central nervous system (CNS) functions. This combination of desirable properties, including reversibility, anatomically restricted effects, and selective sparing of other neuronal functions, has helped foster the widespread acceptance and use of these agents in both veterinary and human medicine.

Throughout the modern history of drug discovery and development, it has been common for the initial lead compound within many drug classes to be identified and isolated from a natural source, and that is certainly the case for the local anesthetics. For many centuries, native peoples living in the Andes mountains of South America chewed extracts of the leaves from the coca shrub (*Erythroxylon coca*) for both stimulatory effects and euphoric properties. However, it was not until 19th century that the chemist, Albert Niemann, reported the chemical synthesis of cocaine, the principal active ingredient in coca plant extracts. The local anesthetic properties of this plant alkaloid became known in Western medicine soon thereafter when Niemann reportedly discovered that this newly isolated compound caused numbness in his tongue when he tasted the synthetic cocaine. The ready availability of synthetic cocaine led to studies by Sigmund Freud into the drug's physiological actions and its eventual introduction into clinical usage for surface analgesia in ophthalmology and eventually for parenteral use to block nerve conduction. As described later in Section Amino-Ester versus Amino-Amide Classes, cocaine is considered to be the archetype for the amino-ester class of local anesthetics and its discovery led to the synthesis and introduction of other amino-ester local anesthetics into clinical use, including benzocaine (1900), procaine (1904), dibucaine (1929), tetracaine (1930), proparacaine (1953), and others. The other broad chemical class of local anesthetics, which are known commonly as amino-amide agents, was brought into clinical use with the introduction of lidocaine in 1948, followed by mepivacaine (1957), prilocaine (1960), bupivacaine (1963), and other agents. The significance of the amino-amide versus amino-ester classifications of local anesthetics is discussed in Section Amino-Ester versus Amino-Amide Classes.

Clinical Uses in Veterinary Medicine

In the clinical setting, most actions of local anesthetics are readily reversible and devoid of any significant effect on an animal's consciousness or other functions that are associated with the CNS. This combination of properties makes these agents very useful for preventing pain that would normally occur during a wide variety of treatments and procedures. Disruption of neural transmission in sensory afferents with a local anesthetic agent can be accomplished in several distinct ways,

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including local tissue infiltration, splash blocks, intravenous regional nerve blocks, digital nerve blocks, or by administration of local anesthetics in close proximity to spinal nerve tracts (epidural and intrathecal administration) in order to disrupt pain transmission into higher CNS centers. When these agents are administered correctly with particular attention to anatomical injection site, injection volume, dose, and coadministration of a vasoconstrictor agent, it is possible to produce complete analgesia in a targeted region of the body. In addition, local anesthetics may contribute further to pain management in patients by interfering with the central facilitation (wind up) and sensitization of nociceptive pathways.

Local anesthetic agents are used clinically in many different ways for local or regional blockade of pain in both small and large animal species. The following listing includes some of the more common applications for local anesthetic use, although some specialized applications are not included here.

Topical anesthesia: In order to be effective for topical anesthesia, the local anesthetic agent must be capable of penetrating through open wounds, skin, or mucous membranes. Penetration of mucous membranes is significant for many local anesthetics and can give rise to significant system absorption, most notably from the respiratory tract. The capacity of most local anesthetics to penetrate intact skin is markedly limited, but certain formulations can be effective for producing superficial dermal anesthesia.

Infiltration anesthesia: These applications require that a high concentration of agent is injected directly into tissue without particular concern for the anatomical location of nerve tracts in the vicinity of the injection site. In view of the requirement for administering a relatively high amount of drug for infiltration anesthesia, there is a greater risk of systemic absorption and toxicity. Such risks are mitigated commonly by inclusion of a vasoconstrictor agent such as epinephrine along with the local anesthetic.

Splash blocks: This clinical use refers to direct application of a local anesthetic to a site of interest, such as proximal to the body wall in association with abdominal surgery or adjacent to ovarian ligaments following ovariohysterectomy. Splash blocks are also used in association with oral surgical procedures, including application into tooth extraction sites. Such diffuse applications of local anesthetics have been shown to reduce general anesthetic requirements in some clinical procedures and are therefore considered beneficial.

Field-block anesthesia: This application commonly involves injection of a local anesthetic immediately adjacent and perpendicular to sensory nerves that innervate a particular region of the body. When performed correctly, local anesthetics used in this manner can effectively ablate all sensations distal to the line of injection sites. In a clinical setting, this particular application requires a detailed knowledge and accurate placement of drug immediately adjacent to sensory nerve tracts that innervate the body region. Nerve block anesthesia represents a more confined application in which the local anesthetic is injected in immediate proximity to a nerve or nerve plexus. The risk of toxicity is considerably less in this application due to more restricted drug placement and the use of limited quantities.

Intravenous regional nerve block (IVRA or Bier block): These clinical applications provide a rapid and reliable method for evoking short-term anesthesia and muscle relaxation of an animal's distal extremities. The blood supply to a distal limb is isolated by a tourniquet and the local anesthetic is injected intravenously distal to the tourniquet. The mechanism(s) by which local anesthetics produce IVRA is unknown but may arise from local diffusion of the agent into tissue and action on nerve endings and nerve trunks. Problems associated with this method include systemic toxicity if the tourniquet leaks or is released before the local anesthetic has become bound to tissue. Harmful effects secondary to prolonged disruption of the blood supply with the tourniquet also present some concern with these applications.

Soaker-type catheter: In this approach, which is commonly referred to as a diffusion or wound catheter, a length of sterile fenestrated tubing is placed surgically at a painful site for the continuous or intermittent administration of local anesthetics. Soaker-type catheters are used in association with limb amputation or large tumor resection or a course of palliative care.

Spinal and epidural anesthesia: With spinal (subarachnoid) anesthesia, local anesthetic is injected into the lumbar-dura space immediately surrounding the spinal cord where it mixes with the cerebrospinal fluid (CSF). Besides producing the block of sensory innervation, the most notable physiological effect is the sympathetic blockade produced in spinal nerve roots. The degree of spinal anesthesia produced by this method is dependent on the injected volume as well as the drug concentration and the extent of drug diffusion along the spinal cord. Since preganglionic sympathetic fibers are very sensitive to local anesthetic blockade, the sympathetic block typically extends in a rostral direction for one or two additional segments. The movement of anesthetics along the neural axis is determined by volume of injection and by patient position. In epidural anesthesia, drug is injected into the space between the ligamentum flavum and the dura mater that covers the spinal cord. The presumptive site of drug action is spinal nerve roots, although some drug is likely to be absorbed into the epidural space where additional effects could take place.

Mechanisms for Local Anesthetic Action

Multiple lines of evidence indicate that the primary cellular target for local anesthetic action is (are) one or more specific binding sites located within ion-specific channels in the plasma membranes of nerves and other excitable cells (see review by Nau and Wang, 2004). When drug binding sites are occupied by a local anesthetic, the channels are unable to conduct Na^+ ions inwardly as normally occurs during periods of intense membrane depolarization. As a consequence of this blockade, the threshold for electrical excitation of the nerve membrane increases, while the rate of rise of the action potential, the speed of impulse conduction, and the safety factor for impulse conduction are collectively reduced. Eventually, as fractional drug occupancy of channel binding sites approaches saturation, the affected regions of nerve cells become incapable of generating or conducting action potentials thereby causing failure of nerve impulse conduction. Results from experimental studies indicate that the locations of local anesthetic binding sites are deep within the Na^+ channel complex and appear to be at or near the axoplasmic (inner) surface of the membrane (Butterworth and Strichartz, 1990). This conclusion is based in part on early experimental observations that quaternary analogs of local anesthetics, which are highly charged at physiological pH, selectively block nerve impulse conduction when applied internally to isolated nerve axons, but are relatively ineffective when applied externally. In keeping with this model, it would seem essential that local anesthetic agents must first permeate the nerve membrane in order to access specific binding sites within Na^+ channels. This and other observations have led to the hypothesis that the site at which local anesthetics act, at least in their charged form, is accessible only from the inner surface of the membrane (Narahashi and Frazier, 1971). It is well-established that voltage-gated Na^+ channels in mammalian neurons are comprised of glycosylated proteins with an aggregate molecular size in excess of 300,000 daltons. The largest of the protein subunits that form the channel complex contains four homologous domains, each containing six alpha-helical transmembrane segments (Figure 15.1). This component of the channel complex is considered to contain the specific binding pocket for local anesthetic agents (Yu et al., 2005). Although several alternative models have been proposed to explain the mechanism for local anesthetic action, including a variety of physicochemical models (Courtney and Strichartz, 1987), none of these hypothetical models are as widely accepted as the Na^+ channel binding site model described above.

Activity-Dependent Blockade

The extent or degree of conduction block that occurs in the presence of a given concentration of local anesthetic depends on several factors, including the resting membrane potential, the degree of nerve stimulation (firing activity), the length of axon in which Na^+ channels are blocked, and the duration of exposure to drug. Local anesthetics display an enhanced capacity to block nerve impulse conduction in nerves that are depolarized and actively firing (frequency-dependent blockade), presumably due to an increased capacity for drug to enter channels that are in the open state (ion conducting) versus an inactivated or nonconducting closed state. Once bound to a channel, a local anesthetic stabilizes Na^+ channels in an inactivated state that is incapable of conducting Na^+ ions (Butterworth and Strichartz, 1990; Courtney and Strichartz, 1987). Some but not all local anesthetic agents exhibit differences in the extent of frequency-dependent nerve blockade owing to differences in the rates of drug dissociation from the channel binding site. For agents that dissociate more rapidly from channel binding sites (small hydrophobic agents), effective channel blockade requires more rapid firing activity in order that the rate of drug binding during the action potential exceeds the rate of drug dissociation from Na^+ channels between action potentials. From a clinical perspective, the impact of this frequency-dependent blockade has limited relevance for sodium channel blockade in sensory nerves, but is considered to be important for Na^+ channel blockade in myocardial tissues, where the antiarrhythmic actions of these agents can be impacted significantly by the relative rates of drug association and dissociation from channel binding sites.

Differential Effects on Nerve Fibers

By virtue of the pervasive presence of voltage-gated Na^+ channels in nearly all nerve cells, it seems likely that local anesthetics would have the capacity to block impulse conduction in most if not all neurons that undergo sodium-dependent firing. Nevertheless, numerous reports from studies in isolated tissues and intact living animals have provided solid evidence for the ability of local anesthetics to exert differential or selective blocking actions on nerve fibers that transmit impulses associated with different sensory modalities or functions. In general, these studies indicate that local anesthetics first cause diminished sensation to pain and temperature along with loss of other sensory modalities

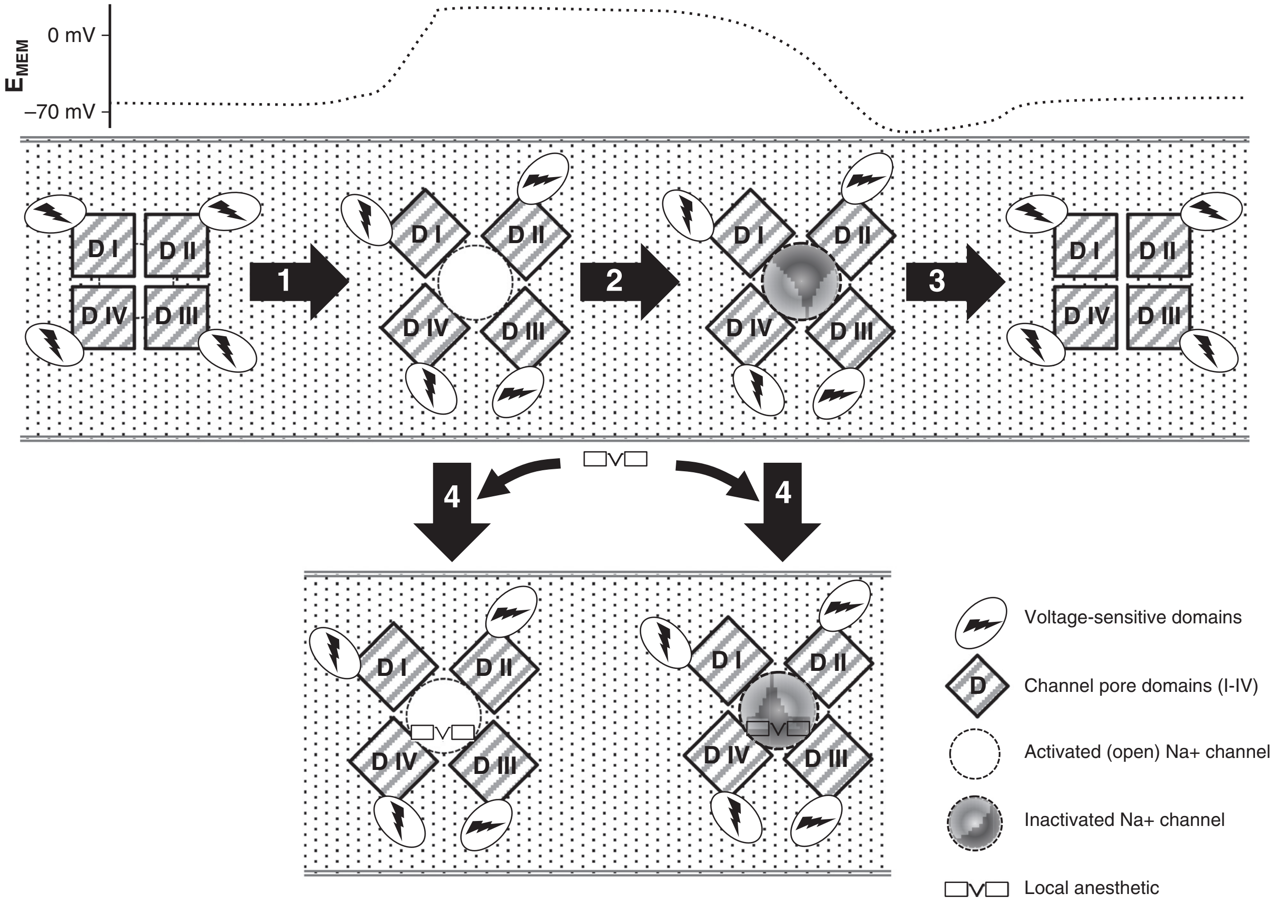

E_MEM
0 mV
–70 mV
1
2
3
4
4
D I
D II
D III
D IV
Voltage-sensitive domains
Channel pore domains (I-IV)
Activated (open) Na+ channel
Inactivated Na+ channel
Local anesthetic

Figure 15.1 Theoretical model for sodium channel blockade by local anesthetics. The four homologous transmembrane domains (DI, DII, DIII, and DIV) that form the pore of the Na^+ channel are depicted as an array of squares from the perspective of looking at the outer membrane surface. Membrane depolarization causes conformational shifting of voltage-sensing domains and opening of the channel pore (step 1). As sodium ions enter through the open channel, membrane potential (E_{MEM}) becomes positive leading to inactivation of the open channel (step 2). Ultimately, the channel returns to a closed state (step 3) and the cycle repeats. Local anesthetics bind to sites on domains III and IV near the inner membrane surface when channels are in an open or inactivated state (step 4). Once occupied by local anesthetic, the channel becomes incapable of conducting Na^+ ions.

(touch and deep pressure), followed by diminished proprioception and motor function. In general, autonomic fibers, small unmyelinated C fibers (mediating pain sensations), and small myelinated A fibers (mediating pain and temperature sensations) are blocked preferentially before larger myelinated fibers that mediate postural, touch, pressure, and motor information. The underlying mechanism(s) that give rise to this differential blockade are incompletely understood, but several factors may contribute, including fiber diameter and extent of axonal myelination. However, the early hypothesis that sensitivity to local anesthetic blockade was inversely correlated to nerve fiber diameter does not fit with all experimental observations (Fink and Cairns, 1984; Franz and Perry, 1974; Huang et al., 1997) and thus it appears unlikely that fiber size per se determines susceptibility to local anesthetic block under steady-state conditions. The spacing between nodes of Ranvier could also contribute to differential sensitivity to local anesthetic action since internodal spacing typically increases in proportion to the diameter of nerve fibers. Because a fixed number of nodes must be blocked in order to prevent impulse conduction, small fibers with closely spaced nodes of Ranvier may be blocked more readily following exposure to local anesthetics. Finally, differences in tissue barriers and the relative locations of smaller C fibers and A fibers within sensory nerve trunks may also contribute to the apparent differences in nerve sensitivities to local anesthetic action. It is important to note that substantial controversy still surrounds the basic premise for differential susceptibility of nerve fibers to local anesthetic action. Since the primary clinical goal of local anesthesia is to alleviate or prevent pain, it would be beneficial if motor functions were spared completely when sensory modalities are attenuated or, at a minimum, that any effect of local anesthetics on motor function and

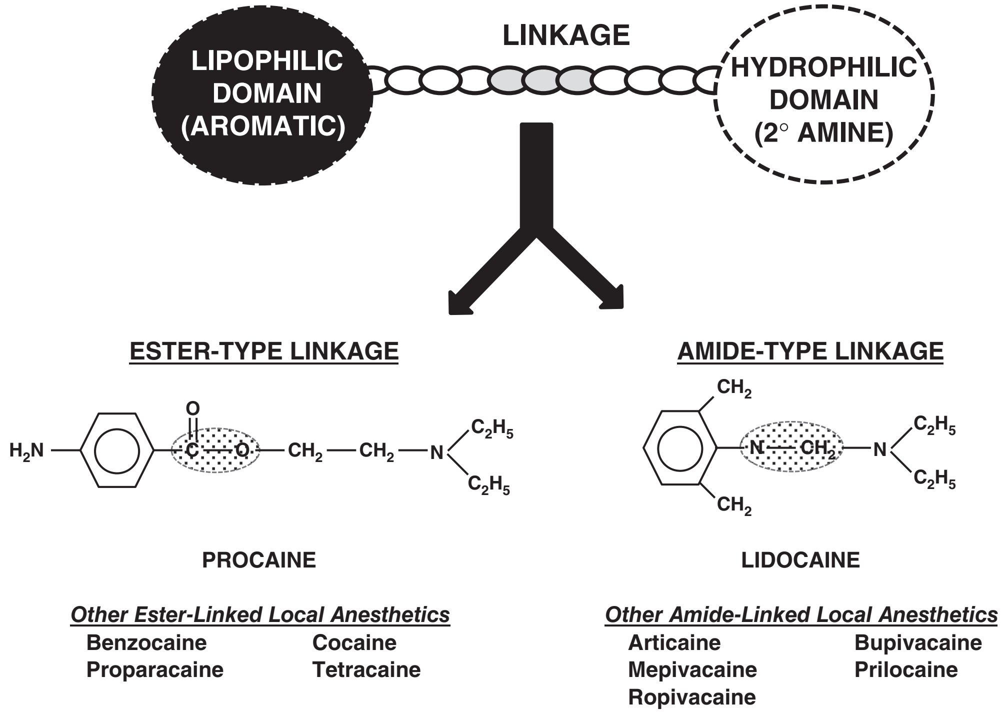


Figure 15.2 Representation of the core structural elements for two major classes of local anesthetic agents. Chemical structures for archetype representatives of the ester-linked agents (procaine) and amide-linked agents (lidocaine) are shown with the carboxylic acid ester and amide chemical bonds, respectively, highlighted within the shaded region.

proprioception would dissipate completely before sensory functions were recovered fully. Unfortunately, this is not always the outcome in clinical practice. A practical and important example in veterinary medicine occurs in horses, where limb nerve blocks are performed to assist with certain clinical procedures or diagnostic tests. In such instances, impaired proprioception increases the risk of injury if the horse is permitted to move about without proper restraint. Such heightened risk of injury is one reason why many racing authorities have banned the use of local anesthetics in horses prior to racing. The long-acting local anesthetic etidocaine was withdrawn from the market owing to problems associated with persistent motor deficits in horses and some other species.

Chemical Properties

Amino-Ester versus Amino-Amide Classes

Local anesthetics are organic bases, but are classified as amphipathic substances since nearly all agents contain both lipophilic (aromatic) and hydrophilic (substituted amine) moieties. These chemically diverse regions of the molecule are linked by an intermediate-length carbon chain that contains either an amide bond or ester bond, which gives rise to the frequent subclassification of local anesthetics into amino-amide and amino-ester classes of agents (Figure 15.2). The distinction between amino-amide and amino-ester local anesthetic agents represents far more than some arcane chemical classification insofar as enzyme-catalyzed disruption of these bonds within the linker regions renders agents incapable of producing a local anesthetic effect and, in many cases, represents a significant route for inactivation. In general, ester-linked agents are highly susceptible to enzyme-catalyzed hydrolysis of the ester bond by different families of esterase enzymes and, as a consequence, tend to have much shorter durations of action as compared with amide-linked agents. This is an important consideration in animals that have a diminished overall level of esterase enzyme activities secondary to disease processes, genetic abnormalities, or, perhaps most importantly, prior exposure to agents that cause reversible or irreversible inhibition of esterase enzymes. By comparison, the amino-amide link is more resistant to enzymatic degradation and is highly stable under temperature extremes, including heat sterilization.

Effect of Local pH

The physical chemical properties and structure–activity relationships for local anesthetic agents have been reviewed in detail previously (Courtney and Strichartz, 1987). Most local anesthetics contain a tertiary amine as the hydrophilic group and an unsaturated aromatic ring as the lipophilic group. In general, tertiary amines are poorly soluble in water and, accordingly, many local anesthetics are prepared as hydrochloride salts in order to improve water solubility and facilitate their formulation as injectable aqueous solutions. Since local anesthetics are weak organic bases (B), with typical pK_a values ranging from 8 to 9, the hydrochloride salts form mildly acidic solutions in water, which tends to enhance the chemical stability of catecholamines, such as epinephrine, that are sometimes included for their vasoconstrictor action. Under usual conditions of administration, it is likely that the pH of the local anesthetic solution rapidly equilibrates with that of the extracellular fluid environment. Experimental studies support a model (Figure 15.3) wherein the unprotonated species of the local anesthetic (B^0) is the predominant form that is able to diffuse across cellular membranes, whereas the less permeable cationic species (BH^+) is the predominant form that interacts preferentially with Na^+ channels. In consideration of this model, wherein the unprotonated form of drug provides improved access to the target site and the protonated form has greater affinity for blocking Na^+ channels, it is evident that pH of extracellular fluid can influence the actions of local anesthetics. The relative concentrations of uncharged (B^0) and cationic forms (BH^+) of drug at equilibrium are related to the pKa of the ionizable group as well as the local pH and can be estimated mathematically by the Henderson–Hasselbalch relationship as follows:

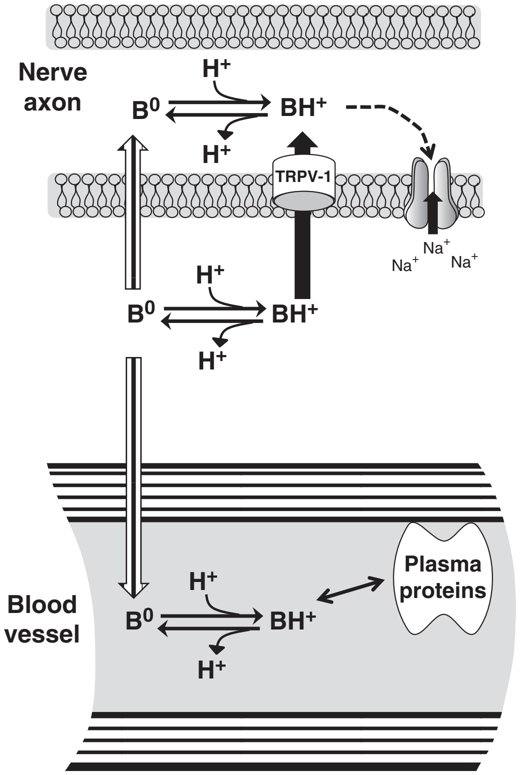


Figure 15.3 Principal routes for local anesthetic movement following subcutaneous injection. Passive diffusion (thick striped arrows) is likely to be the primary process by which the unprotonated (B^0) forms of local anesthetics enter either nerve axons or blood vessels. In addition, the cationic form (BH^+) may enter neurons via transient receptor potential vanilloid subtype-1 channels (TRPV-1), if present, and allow for blockade of sodium channels from the axoplasmic side.

$$pH = pK_a + \log \frac{[B^0]}{[BH^+]} \tag{15.1}$$

When solution pH is equivalent to the pKa of a local anesthetic, the relative concentrations of $[B^0]$ and $[BH^+]$ are equal. However, in situations where the extracellular fluid becomes mildly acidified (reduced pH), such as in association with localized tissue inflammatory reactions, the relative ratio of $[B^0]/[BH^+]$ decreases in accordance with the above relationship and the cationic form becomes the predominant species. A modest shift in the pH of extracellular fluid can cause a significant shift in the equilibrium between cationic and neutral forms and thereby affect the rate at which drug is able to diffuse through the nerve cell membrane and reach the target binding site. Another situation where the equilibrium concentrations of charged species could be important is with regard to local anesthetic diffusion across the maternal–fetal barrier in the placenta, where fetal blood is slightly acidic (lower pH) relative to maternal blood. However, it is important to recognize that the impact from shifts in the acid–base equilibrium can be influenced by other factors for certain agents, including differences in drug–protein binding, differences in the membrane permeability of neutral and cationic species, as well as differences in the Na^+ channel blocking activity for the neutral and cationic species. For example, it has been reported that the unprotonated (B^0) form of some local anesthetics can exhibit significant channel-blocking activity, whereas the uncharged form of other agents are nearly devoid of activity. In addition, the capacity for the charged cationic form of local anesthetics to gain access to the cytoplasmic surface of the nerve cell membrane by passage appears to not be limited by transmembrane diffusion of the unprotonated neutral form as commonly proposed. For example, research studies have demonstrated that a permanently charged derivative of lidocaine has the ability to permeate neuronal membranes by passage through a family of nonselective cation channels known as transient receptor potential vanilloid subtype 1 or TRPV1 channels (Binshtok et al., 2009). These and related membrane channels are expressed primarily within sensory neurons and, when activated by membrane depolarization, permit influx of inorganic cations, primarily Ca^{2+} and Na^{+}.

However, in light of the selective expression of TRPV1 channels in a subset of sensory neurons, this alternate pathway for local anesthetic entry and blockade of axonal Na^{+} channels (Figure 15.3) raises the potential to achieve greater pharmacological selectivity and thereby avoid unwanted effects on motor nerves and possibly other nonnociceptive modalities (Blumberg, 2007). In addition to being the responsive element for changes in molecular charge under different conditions of pH, the hydrophilic moiety is considered crucial for influencing drug binding affinity to target sites within Na^{+} channels. Structure–activity studies indicate that the intermediate linking chain is likely to be important for proper alignment of the lipophilic and hydrophilic ends of the drug, with a length of three to seven carbon atoms being optimal for channel blocking activity. In general, detailed studies of the structure–activity relationship for local anesthetics have revealed that: (i) highly lipophilic agents exhibit higher potencies, which may reflect an increased binding affinity for target sites as well as enhanced partitioning into the membrane microenvironment; (ii) highly lipophilic agents exhibit longer durations of action possibly due to enhanced distribution into nerve cell membranes plus reduced accessibility to degradative enzymes in plasma and liver; (iii) higher lipophilicity is associated with a greater propensity for toxicity and a smaller therapeutic index; and (iv) agents that are smaller in size exhibit faster rates for dissociation from target receptor sites and, accordingly, display a greater extent of frequency- and voltage-dependent actions in rapidly firing neurons.

Stereoisomerism

Several of the local anesthetic agents that are used clinically, notably those that contain a pipcolyl xylidine (PPX) group within the hydrophilic region, can exist as pairs of optical stereoisomers. As readers may recall from general chemistry, stereoisomers are compounds that have identical chemical structures but differ in the three-dimensional spatial arrangement of constituent groups around a particular atom (usually a carbon atom), which represents the chiral center. The different spatial orientations of stereoisomers for local anesthetics form non-superimposable mirror images of one another that are known as enantiomers. A useful analogy for visualizing differences between chemical enantiomers is to consider enantiomer pairs to be similar to a person's right and left hands. When the right and left hands are oriented with the palms facing in the same direction, they are not superimposable even though they contain the same array of fingers (constituent groups). Enantiomers are most commonly designated as R(+) or *dextro* and S(-) or *levo* and, in the case of local anesthetics, certain enantiomers are known to produce pharmacological actions that are both quantitatively and qualitatively different. More specifically, the S(-) enantiomers of several local anesthetics have been reported to produce fewer adverse effects in the central nervous system (CNS) and the cardiovascular system (CVS) as compared to the R(+) enantiomers of the same drugs. In support of these observations, enantiopure preparations of S(-) isomers have been found to exhibit improved safety profiles relative to the racemic (50 : 50) mixture of enantiomers that are formed during routine chemical syntheses. Accordingly, enantiopure preparations of the S(-) stereoisomer of the local anesthetic bupivacaine have been developed and marketed, as described in Section Levobupivacaine.

Stability and Formulations

Local anesthetics that are used clinically are chemically quite stable substances with aqueous solutions being remarkably stable at ambient temperatures (15–30°C). In the case of lidocaine, aqueous solutions are known to withstand autoclave sterilization (15 PSI at 121°C for 15 minutes), although it should be noted that some local anesthetic formulations contain the catecholamine epinephrine, which readily undergoes spontaneous oxidation under extreme conditions. Such formulations should be protected from elevated temperatures as well as direct sunlight in order to preserve added catecholamine. Formulations that include epinephrine are packaged and sold in amber-colored containers to afford protection against photochemical degradation, but still have a reduced shelf life compared to formulations without epinephrine. Like any other injectable drug solution, solutions of local anesthetics that become cloudy, discolored, or contain visible precipitates should be discarded.

Local anesthetics are available in a wide array of formulations, a fact that underscores the need for careful attention to product labels. Lidocaine, the most widely used local anesthetic in veterinary practice, is available as sterile injectable solutions for parenteral use, gels and ointments for topical use, aerosol sprays for airway application, and patches for transdermal application. Multi-dose injectable formulations routinely contain preservatives that have been linked to adverse actions, including sodium metabisulphite (neurotoxicity), methylparaben (hypersensitivity reactions), benzyl alcohol (intoxication in felines), and EDTA (transient tetany). Preservative-free preparations are available and essential for intrathecal injection owing to the high potential for nerve damage by preservatives. On a related matter, it is interesting to note that the specific gravity (density) of local anesthetic formulations can be a critical factor in spinal anesthesia. In order to restrict the movement of drug solution within the subarachnoid space, anesthesiologists use solutions of local anesthetics that are rendered hyperbaric (specific gravity ≫1) by the addition of a high concentration of glucose. Human formulations of hyperbaric solutions are currently available, although use of these agents is typically limited in veterinary practice. There is little evidence for acute allergic reactions to local anesthetics in humans or nonhuman species and therefore this poses little concern.

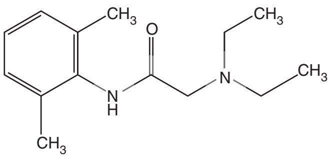
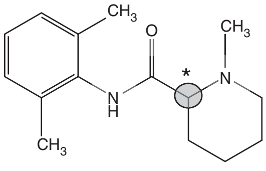
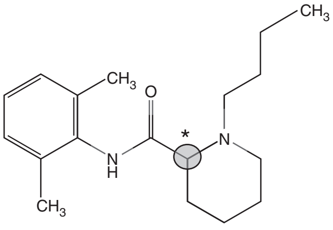
General Pharmacological Considerations

In addition to their shared mechanism of action and core chemical structures, most local anesthetics exhibit an overlapping set of pharmacological actions, including similarities in their pharmacodynamic actions and pharmacokinetic properties. While differences or unique properties of selected individual agents are summarized later in this chapter, it is important to consider properties that are shared among many of the local anesthetics that are used routinely in clinical practice.

Onset and Duration of Action

The amount or dose of local anesthetic that is required to block sensory transmission is highly dependent on the free (unbound) concentration of drug in the cationic form that is available at the channel binding site. Higher lipid solubility translates into greater potency or lower effective dose but, unfortunately, also correlates with organ system toxicity, especially within the cardiovascular and central nervous systems. For this reason, the more potent local anesthetic agents tend to have reduced margins of safety. Since these agents are administered at relatively high concentrations and, in most situations, are used to produce local actions, the relative potencies of local anesthetics are generally considered to be of lesser importance than may be the case for other types of agents that exert systemic actions at sites far removed from the administration site. The time delay between administration of a local anesthetic and the resultant sensory block is influenced by many factors, including proximity of drug placement relative to target sensory nerves, the concentration of drug, and certain physical–chemical properties of the drug, including molecular weight (size) and the relative fraction of drug in the charged/neutral form. Predictably, the closer a local anesthetic is administered to an intended site of action and the higher the concentration of drug that is used will lead to faster onset times. With respect to physical–chemical properties, the pKa is a major determinant of the proportion of drug that is charged or uncharged in any solution, including body fluids. Since the uncharged (base) form of drug is more lipophilic and therefore better able to diffuse into the plasmalemmal membrane compartment of nerves, drugs with lower pKa values tend to have more rapid onset of action. For example, lidocaine and mepivacaine have lower pKa values than bupivacaine (Table 15.1) and, accordingly, exhibit more rapid rates of onset. Lower pKa values have a downside insofar as agents exist primarily as insoluble bases, such as benzocaine (pK = 2.5) where limited solubility renders it useful only for topical mucosal applications in most species, except fishes, where the drug's excellent tissue penetration provides significant benefit as an anesthetic agent.

Table 15.1 Comparative physical–chemical properties and pharmacological properties of three amino-amide class local anesthetics

Property	Lidocaine	Mepivacaine	Bupivacaine
Chemical structure			
	CASN 137-58-6	CASN 96-88-8	CASN 2180-92-9
Stereoisomers	No	Yes	Yes
pKa	7.8	7.7	8.1
Partition coefficient (λ) (octanol : water)	110	42	560
Water solubility (mg/l)	4100	7000	9.2×10^{-5}
Plasma protein binding	64%	80%	90%
Onset (~ min)	5–10 (fast)	10–20 (rapid)	20–30 (slow)
Duration (~ min)	90	130	360

* Indicates location of chiral center in mepivacaine and bupivacaine.

Lipophilicity and protein binding tend to reduce the ability of drugs to permeate nerve membranes and thereby delay the onset time for local anesthetic action. The duration of sensory nerve blockade by most agents is determined by multiple factors that are both extrinsic as well as intrinsic to the nerve cell membrane. Intrinsic factors within the nerve membrane microenvironment include the temporal profile of drug concentration at sodium channel binding sites, the balance between cationic and neutral forms of the drug, as well as the rates of drug association and dissociation from channel binding sites. Lipid solubility of individual agents is a primary determinant for drug distribution into membranes and directly influences the ability of local anesthetics to achieve effective concentrations at target binding sites. In addition, the binding affinity between individual agents and sodium channel subunits is critically important for determining duration of nerve blockade, since higher affinities are associated with slower rates of dissociation for the drug–receptor complex. As noted above, drug binding affinity to sodium channels also contributes to differences among individual agents with respect to the kinetics of channel blockade and nerve firing rate. In general, agents that display faster rates of dissociation from Na^+ channels have a greater impact on rapidly firing neurons compared to agents with slower rates of dissociation from channel binding sites.

In addition to intrinsic processes that influence the time course for local anesthetic action, other factors extrinsic to the nerve membrane can have a substantial impact on local anesthetic action. For most local anesthetics, contact time with sensory nerves is of primary importance and is directly dependent on local blood flow. Passive diffusion of local anesthetic into blood vessels in the vicinity of the injection site(s) represents the primary pathway for drug removal and thereby determines the time course for drug exposure to nerves and other potential target sites. As pointed out in Section Clinical Uses in Veterinary Medicine, local blood flow can be, and frequently is, manipulated intentionally by coadministration of vasoconstrictor agents, which reduce regional blood flow and thereby prolong the period of anesthetic action. In clinical practice, epinephrine is used most commonly as a vasoconstrictor agent and is included in some commercial preparations at a low concentration (typically 5 parts per million or 1 : 200,000). In practical terms, vasoconstrictors serve two purposes by reducing drug movement into the vascular compartment and reducing the risk of systemic toxicity. In this way, drug residence near the intended site for action is prolonged and the rate of drug metabolism is better able to keep pace with drug absorption into the circulation.

In conjunction with these benefits, there is some potential for added risk from the use of vasoconstrictors, including untoward cardiovascular changes and other reactions associated with excessive systemic absorption or accidental intravascular injection of the vasoconstrictor agent. In addition, there is some potential for delayed wound healing, tissue edema, tissue hypoxia, and necrosis when local anesthetic/vasoconstrictor preparations are administered in multiple sites or within digits, limbs, or other body regions that have limited collateral circulatory supplies. For this reason, vasoconstrictors are contraindicated in applications (e.g., ring blocks) where there is risk of compromised blood supply. One final concern, relates to the administration of epinephrine in body regions where blood vessels are preferentially dilated rather than constricted by mixed-acting adrenergic agents (i.e., mixed α/β agonists) such as epinephrine. In tissues such as skeletal muscle, the predominant effect by epinephrine on vascular smooth muscle is relaxation, which is a direct consequence of an overriding response to β_2-receptor activation (relaxation) versus α_1-receptor activation (contraction). Under such conditions, local blood flow is increased and systemic absorption of local anesthetic may also be increased, thereby increasing the risk for adverse effects. In order to avoid this unwanted consequence, epinephrine should either not be used or replaced with a different vasoconstrictor agent, such as phenylephrine, that preferentially stimulates α_1-adrenergic receptors and presents little risk for a vasodilatory action. Several local anesthetics that are used clinically have some capacity to cause vasoconstriction of their own accord, including bupivacaine and ropivacaine. Cocaine, the classical local anesthetic agent, is the most potent vasoconstrictor local anesthetic owing to its ability to competitively inhibit catecholamine transporters and potentiate the actions of most endogenous catecholamines, including epinephrine and norepinephrine.

General ADME Considerations

As local anesthetics are absorbed from the extracellular fluid at injection sites, agents are subject to several potential fates as they enter the systemic circulation. Once in the bloodstream, some local anesthetics undergo extensive binding with plasma proteins, notably α_1-acid glycoprotein and, to a lesser extent, serum albumin. Local anesthetics exhibit high binding affinity with the former, but the capacity is low and can be saturated readily. In contrast albumin provides a large capacity for drug binding, but binding is lower affinity and may have limited relevance except at very high drug concentrations. While the plasma protein binding properties of individual local anesthetics have not been fully delineated across most animal species, based upon human data it is suspected that agents in the amino amide class are highly bound to plasma proteins(up to 95%) whereas most ester-linked local anesthetics are not highly bound. For amino-amide agents such as bupivacaine and ropivacaine, where the protein-bound fractions of drug are high, small reductions in protein binding hold the potential to produce significant adverse consequences owing to the corresponding large shift in the fraction of free drug. As true for any drug that is highly bound to plasma protein, binding of local anesthetics may be influenced through several possible means, including competitive displacement by drugs or endogenous substrates that bind with high affinity to the same classes of serum proteins, changes in plasma pH that cause a shift in drug–protein interactions, or changes in serum proteins associated with conditions or diseases that affect levels of plasma proteins per se. For example, several factors have been reported to increase circulating levels of α_1-acid glycoprotein in humans (cancer, surgery, trauma, myocardial infarction, and uremia), and it is possible that similar changes may take place in nonhuman species. Since most systemic actions of local anesthetics are unwanted and considered adverse in a clinical setting, it is prudent to consider such factors that can impact the free concentration of diffusible drug in clinical patients.

Metabolic degradation of local anesthetics is arguably the preeminent consideration with respect to potential adverse effects by local anesthetics that are used in routine veterinary practice. In general, adverse reactions to local anesthetics are closely associated with circulating drug concentration, which is a direct reflection of the balance between the relative rates of drug absorption and drug elimination. Once absorbed from an injection site, local anesthetics are subject to enzyme-catalyzed metabolism or available to diffuse into tissues where the drug may produce untoward reactions. For this reason, care must be taken to avoid direct intravascular administration of a high concentration of local anesthetic, since the rapid intravascular delivery is likely to overwhelm metabolic processes and pose substantial risk of adverse effects. For agents that contain an amino-ester linkage (i.e., cocaine, benzocaine, procaine, and tetracaine), enzymatic metabolism most commonly follows initial catalytic hydrolysis of the ester link by different families of esterase enzymes, including plasma cholinesterases, acetylcholinesterase, and nonspecific esterases in liver and other tissues. In all cases, it appears that hydrolysis of the amide-ester linkage causes loss of local anesthetic activity. In animals with deficient or abnormal esterase enzyme activities or following exposure to agents that inhibit these enzymes, amino-ester linked local anesthetics can display more intense or prolonged actions. In the case of local anesthetic injections into the intrathecal space, amino-ester local anesthetics can exhibit prolonged actions since CSF normally contains no esterase enzymes and termination of drug action relies primarily on absorption back into the general circulation.

It is important to note that one of the by products from procaine metabolism is para-aminobenzoic acid (PABA), which has the potential to compete with and attenuate the antimicrobial actions of sulfonamide antibiotics. In addition, procaine may provoke allergic reactions in animals that are sensitive to sulfonamide-class agents. In contrast to the ester-type agents, metabolism of the amino-amide class of local anesthetics occurs through hepatic biotransformation, although specific pathways have not been fully elucidated in all domestic species.

The most common pathway for hepatic metabolism of amino-amide local anesthetics involves initial *N*-dealkylation followed by additional metabolic transformations. Lidocaine metabolism is perhaps best described and proceeds via oxidative dealkylation by liver cytochrome P450 (CYP) enzymes to form monoethylglycinexylidide (MEGX) and glycinexylidide (GX) as well as additional secondary products. Cleavage of the amide bond in agents that contain a pipecolyl moiety (mepivacaine, bupivacaine, and ropivacaine) proceeds at a slower rate due to chemical hindrance by the bulky alkyl groups surrounding the piperidine nitrogen, which gives rise to the longer biological half-lives for these agents. In view of the importance of hepatic metabolism for elimination of the amino-amide local anesthetics, animals with liver disease may exhibit a reduced capacity to eliminate these agents. Metabolic handling and excretion of urinary metabolites of several local anesthetics has been studied in horses owing to regulatory concerns related to use of these agents in racing animals (Harkins et al., 1995). Urinary excretion of unmetabolized drug appears to be limited and variable and has been reported to account for only 2–5% of lidocaine and bupivacaine and slightly more than 10% of mepivacaine in humans.

Drug Interactions

The potential for drug interactions is an important consideration in the decision to use or selection of a local anesthetic agent. While some interactions are undesirable, not all are problematic. Many interactions are agent specific whereas others appear to be more general for the entire class of agents. Pharmacological interactions between local anesthetics and other drug classes include both pharmacodynamic and pharmacokinetic interactions and, in many cases, are reliant on systemic drug levels that are associated with systemic administration, such as constant rate infusion rather than the more typical route(s) used to produce a local anesthetic effect. Among the most studied and documented interactions is the capacity of lidocaine to lower the required general anesthetic doses of isoflurane in cats, dogs, and horses (Himes et al., 1977; Doherty and Frazier, 1998; Muir et al., 2003).

Organ Toxicities

In view of the capacity of local anesthetics to exert actions on voltage-activated sodium channels in cell membranes of excitable tissues, it is not surprising that the major adverse effects of these drugs occur within organ systems that are highly dependent on cellular electrical activity, namely the central nervous and cardiovascular systems. As depicted in Figure 15.4, signs of local anesthetic-induced adverse effects progress from initial effects in the CNS and, at higher plasma concentrations, expand to involve other organ systems, notably the CVS. The most effective means to diminish the risk for local anesthetic toxicity is to take steps to maintain a low plasma drug concentration and, in that regard, the most important consideration is dose. The amount of drug administered and the time frame over which it is given is a primary risk factor for local anesthetic toxicity and is likely why toxicity occurs more frequently in small animals, in which recommended dose limits may be exceeded when drug is administered repeatedly in small increments. As discussed previously, steps should be taken to minimize vascular absorption, including use of vasoconstrictors, avoiding highly vascularized sites when practicable, and taking care to avoid direct intravascular drug administration. In clinical approaches where there is deliberate intravascular injection (e.g., IVRA), toxicity can be avoided as long as sufficient time is allowed for drug to bind with tissues before the tourniquet is released. Care must be used with local anesthetic injections into the intercostal space since this region is highly vascularized and provides a site for rapid drug absorption.

Central Nervous System

The ability of local anesthetics to produce rapid adverse neurological effects is a major concern. In most cases, local anesthetics produce generalized descending CNS depression that is proportional to the free (unbound) circulating concentration of drug. The earliest signs in animals frequently involve subjective minor behavioral changes followed by muscle twitching and tremors that may occur initially in facial musculature (twitching of lips, movements of whiskers, etc.) but spread rapidly to become full tonic–clonic convulsions. Although the sequence of adverse signs differs somewhat among various agents, the sequence of events is suspected to follow effects that arise initially from brain regions that are most highly vascularized (limbic regions) and equilibrate with drug in plasma most quickly. In the case of lidocaine, initial twitching and convulsions is believed

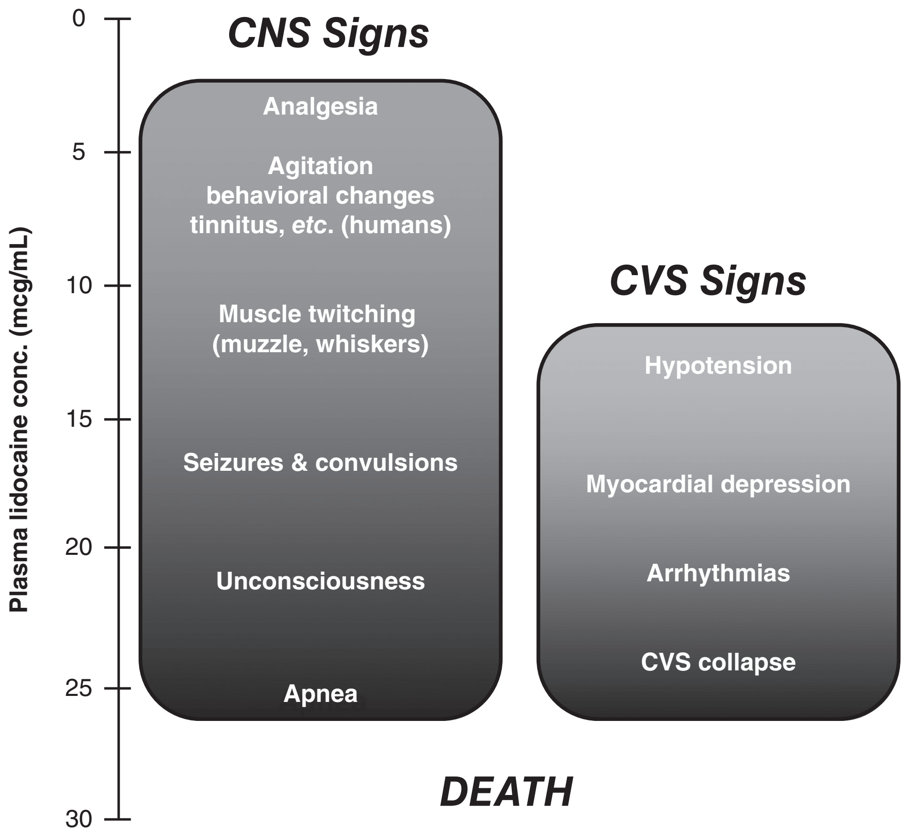


Figure 15.4 Representation of adverse effects from lidocaine in the central nervous system (CNS) and cardiovascular system (CVS) as a function of plasma drug concentrations in the dog.

to arise from initial preferential blockade of inhibitory pathways, rather than any direct excitatory actions that can occur with cocaine. Differences in the acute toxicities of individual agents are likely a reflection of differences in the concentration of free drug that is achieved within the CNS, which is dependent on the rate of drug passage through the blood–brain barrier as well as the rate of drug metabolism. Metabolism tends to be less important for amide-linked agents but can be a significant factor for the ester-linked local anesthetics, which are readily metabolized by plasma esterases. In general, the greater lipophilicity and higher plasma protein binding of amide-linked agents, particularly bupivacaine, can give rise to blunted and transient peak plasma drug concentrations, which may explain in part why such agents are less likely to produce adverse CNS signs initially. If CNS signs occur following local anesthetic administration, symptoms are managed through various means, including a higher concentration of supplemental oxygen and intravenous administration of a benzodiazepine or other anticonvulsant agent in order to control seizures.

Cardiovascular System

Cardiovascular toxicity by local anesthetic agents is complex and involves direct mechanical and electrophysiological effects on the myocardium as well as direct and indirect effects on physiological processes that control peripheral circulation. The primary adverse signs of local anesthetic effects in the CVS are a drop in blood pressure with reduced tissue perfusion and cardiac dysrhythmias. As shown in Figure 15.4, CVS changes caused by lidocaine develop at higher plasma drug concentrations as compared to adverse CNS signs, although that is not the case for racemic bupivacaine, which is substantially more cardiotoxic than lidocaine. With regard to lidocaine, severe hypotension arises from several underlying causes, including depressed myocardial contractility with reduced cardiac output, direct relaxation of vascular smooth muscle, and a generalized loss of vasomotor sympathetic tone. Enhanced cardiovascular sensitivity to bupivacaine appears to derive in part from its higher lipophilicity and different binding kinetics to cardiac sodium channels. Electrophysiological studies have revealed that bupivacaine binds more rapidly than lidocaine with open myocardial sodium channels during membrane depolarization associated with an action potential (systole). However, unlike lidocaine, which dissociates from channel binding sites during diastole, bupivacaine remains bound owing to a slower rate of dissociation from the channel complex. Accordingly, as heart rate increases, a greater fraction of channels remain occupied by bupivacaine, giving rise to significant electrophysiological consequences. The basis for myocardial toxicity by bupivacaine is shared with etidocaine, which causes similar problems from reentrant arrhythmias. In animals that develop cardiovascular depression or collapse, clinical management requires both ventilatory and cardiovascular support, which may include atropine in animals with severe bradycardia. Several controlled trials have indicated that the success rate for resuscitation of dogs suffering from bupivacaine-induced cardiac collapse is substantially less than dogs in which the causative agent is lidocaine (Groban et al., 2001; Picard and Meek, 2006).

Regulatory Considerations

At present there are only three products that are granted approval by the Food and Drug Administration (FDA) Center for Veterinary medicine (CVM) for specific use as local anesthetics in nonhuman species. Mepivacaine (Carbocaine-V®, NADA 100-703), is approved for use in horses, but not in horses intended for human consumption. Proparacaine (Ophthaine®, NADA 009-035), has the unusual designation of "unspecified (animals)" as the target animal species. This unspecified designation is a holdover from the former approval system that is still in place for products that were approved many years ago. Lidocaine is the only other local anesthetic that has been granted FDA-CVM approval for use in dogs (Lidocaine Hydrochloride with Epinephrine Injection 2%, NADA 045-578), although that particular product was withdrawn voluntarily by the sponsor many years ago. There are several approved veterinary products in which local anesthetic agents are formulated in combination with other active ingredients, notably procaine, tetracaine, and lidocaine. However, none of these products are indicated for specific use as local anesthetics in the approved species. Combination products that contain procaine and penicillin G are known to be a source of problems in performance and racing animals owing to regulatory testing for procaine as a regulated substance. Local anesthetics are classified as Class 2 substances by the Association of Racing Commissioners International (ARCI) owing to their potential for masking pain. Detection of these drugs or their derivatives in postrace urine samples can result in substantial penalties, including fines, suspensions, and license revocation. The absence of any FDA-approved local anesthetics for use in food-producing animal species is somewhat problematic, since there are many occasions where such agents are needed to control pain. While this remains one of many areas that is not addressed specifically by any federal statute, the FDA regards the use of local anesthetics in food-producing species to be of low regulatory concern. Nevertheless, since the use of any local anesthetic in a food animal constitutes extralabel drug use, such use must be accompanied by a scientifically derived extended withdrawal time to assure the absence of any unsafe residues in human food products. In view of the relatively short half-life of lidocaine in most species, the Food Animal Residue Avoidance Databank (FARAD) has recommended withdrawal intervals of 24 hours in meat and milk following epidural administration (up to 15 ml of 2% solution) in cattle and longer withdrawal intervals of 72 hours (milk) and 96 hours (meat) following subcutaneous infiltration of lidocaine (inverted L block, up to 2 g) in cattle. These recommendations are based in part on a study by Sellers and colleagues (2009). The following discussions of individual local anesthetics focus on agents that are used most commonly in clinical veterinary practice.

Lidocaine

Lidocaine is the original and prototypical amino-amide local anesthetic that continues to be the most widely used local anesthetic agent in veterinary practice. The widespread popularity of lidocaine is attributable to several pharmacological and nonpharmacological features, including its low cost, long shelf life, chemical stability, heat tolerance, and availability in a multitude of therapeutic formulations. Lidocaine possesses a good pharmacological profile, including rapid onset (2–5 min), an intermediate duration of action (20–40 min), and lower systemic toxicity than bupivacaine. In animals that are sensitive to amino-ester local anesthetics, lidocaine is the agent of choice. Lidocaine is absorbed rapidly following most routes of administration and, therefore, it is combined frequently with epinephrine or another vasoconstrictor agent in order to reduce intravascular absorption of drug when administered by a nonvascular route. In addition to its well-recognized actions as a local anesthetic, lidocaine has been used clinically in both small and large animal species to treat ventricular tachyarrhythmias (Class 1B antiarrhythmic agent) and has been investigated for potential therapeutic uses to mitigate tissue reperfusion injury associated with hypoxia, especially in the treatment of equine postoperative ileus as well as other possible applications for gastrointestinal disorders (Cook and Blikslager, 2008). Several additional applications for lidocaine remain largely investigational at this time although they hold good promise for expanding the clinical use of lidocaine, including treatment of inflammation secondary to multiorgan dysfunction syndrome (Cassutto and Gfeller, 2003), generalized control of pain in dogs, cats, and selected large animal species, as well as adjunctive use for decreasing the requirement for general anesthesia, notably inhalation anesthetic agents. Although there are limited evidence-based clinical benefits that have been demonstrated for these or other uses of lidocaine, this drug remains the subject of significant clinical interest and research. Many of these and other therapeutic applications of lidocaine are addressed in greater detail elsewhere in this text and other publications.

The metabolic fate and pharmacokinetics of lidocaine has been investigated in many nonhuman species. In general, lidocaine undergoes rapid hepatic aminodealkylation by CYP enzymes, which gives rise to relatively short terminal half-lives of less than 1 hour for the parent drug in most domestic species. Hepatic metabolism of lidocaine has been described in great detail in humans and dogs wherein there is an initial formation of two major metabolites, notably monoethylglycine xylidide (MEGX) and glycine xylidide (GX), which consequently undergo additional metabolic transformation into monoethylglycine and xylidide, respectively. In humans, approximately three-fourths of the xylidide metabolite is excreted in urine as 4-hydroxy-2, 6-dimethylaniline. Both MEGX and GX retain some capacity to block sodium channels and, thereby, may contribute to the spectrum of pharmacological effects produced by lidocaine, especially in situations where it is administered over a prolonged period of time (e.g., constant rate infusion) that may cause significant accumulation of active metabolites. While the clinical relevance of pharmacological actions by lidocaine metabolites remains unclear, several studies point to new possible roles or applications in humans and nonhuman species. A study in cultured glial cells from the CNS of rats has revealed a novel action for both MEGX and GX, specifically their ability to act as potent inhibitors of glycine transport. In view of the major inhibitory role of glycine as a CNS neurotransmitter, this effect by lidocaine metabolites has been postulated to contribute in part to the analgesic actions produced by systemic lidocaine administration (Werdehausen et al., 2012). In addition, the rate of hepatic lidocaine clearance and formation of MEGX from lidocaine has been demonstrated to provide a dynamic liver function test in humans with liver disease or reduced hepatic blood flow and has been evaluated for similar application in dogs (Neumann et al., 2011; Pérez-Guillé et al., 2011). Therefore, although lidocaine is one of the oldest local anesthetic agents that remains in clinical use, there may yet be more that can be learned from continued studies of the actions of this old mainstay drug.

At plasma concentrations in the low microgram per milliliter range, lidocaine causes acute adverse signs in most species, including humans. While there appear to be some species differences with regard to plasma lidocaine levels that give rise to these signs and symptoms, the sequence and progression of lidocaine toxicity appears fairly similar in humans and many domestic animal species. Initial signs of lidocaine toxicity appear as CNS toxicity and are reported in humans to include drowsiness, tinnitus, disturbances in taste (dysgeusia), dizziness, and muscle fasciculation (twitching). While the latter sign is the only overt symptom that may be readily apparent in nonhuman species, some of the nonspecific behavioral changes attributed to lidocaine toxicity may be a reflection of CNS signs, which frequently dissipate within several minutes when associated with a single bolus injection of lidocaine. In dogs and horses, the window of safety between plasma levels that are effective in controlling cardiac arrhythmias and those that cause acute CNS signs is fairly narrow, and has been reported to be two to threefold. At higher plasma lidocaine concentrations, CNS signs become more pronounced and are accompanied by signs of cardiovascular toxicity, including hypotension and myocardial depression. As the circulating levels of lidocaine increases, seizures, coma, and respiratory depression and arrest will occur. As noted above, the lidocaine metabolites MEGX and GX may contribute in part to these side effects, especially when lidocaine is administered over a prolonged period.

Among local anesthetics that are used in veterinary medicine, lidocaine is noteworthy for being administered by the greatest diversity of administration routes. The diverse uses of lidocaine are likely attributable in part to the wide array of human drug formulations that are available as both prescription and over-the-counter products. Although there are a very limited number of lidocaine products that are approved specifically for nonhuman species, veterinary practitioners have used the full spectrum of human lidocaine formulations that have been developed for topical, ophthalmic, mucosal, and transdermal uses in humans. For topical applications, lidocaine is considered to be most effective when applied to mucous membranes and far less effective on intact skin. Nevertheless, a combination product that contains equal concentrations (2.5%) of lidocaine and prilocaine (a eutectic mixture of local anesthetics or EMLA®) has been used to improve drug penetration through intact skin in several companion animal species (Erkert and MacAllister, 2005), although some problems have been reported in cats. Other formulations for topical application include gels, ointments, viscous solutions, and sprays. Perhaps the most widely studied means for topical administration of lidocaine are human products designed for transdermal delivery (e.g., Lidoderm patch). Pharmacokinetic analyses have been carried out with transdermal lidocaine patches in several companion animal species, including dogs (Ko et al., 2007), cats (Ko et al., 2008), and horses (Bidwell at al., 2007). Although transdermal delivery of lidocaine in horses has been reported to be limited, a report has suggested some novel approaches for enhancing delivery in this species (Stahl and Kietzmann, 2014). In spite of the disparate and somewhat contradictory findings regarding the efficacy of lidocaine following topical application, this approach continues to be used fairly routinely for postsurgical pain relief and other applications.

Bupivacaine

Bupivacaine is among the most potent and longest acting of the amino-amide local anesthetics that are used in veterinary practice. Along with levobupivacaine, mepivacaine, and ropivacaine, all of which contain a pipecolyl xylidide (PPX) moiety, bupivacaine is a highly lipophilic agent, a property that likely contributes to its high potency and extended duration of action relative to lidocaine. Owing to the presence of a chiral center within the PPX moiety, bupivacaine exists as stereoisomers that are designated as S(-) and R(+) isomers, respectively. Bupivacaine is frequently the drug of choice in situations that require use of a long-acting agent, even though it has a slow onset relative to lidocaine, with complete blocks taking 30 min or longer. The longer duration of action (5–8 hours) coupled with a possibly lower tendency to produce motor inhibition makes it advantageous for infiltration anesthesia, nerve blocks, intrapleural administration, as well as spinal and intrathecal techniques. In female human patients, it has been reported that both bupivacaine and its less toxic S(-) stereoisomer, levobupivacaine, appear in breast milk at concentrations approximately one-third of plasma drug concentrations following epidural anesthesia (Bolat et al., 2014). In view of the propensity of bupivacaine to produce adverse cardiovascular effects, which is a significant concern in human patients, this agent is contraindicated for use in IVRA applications, where there may be a significant risk of a rapid increase in plasma drug concentration. Bupivacaine is relatively ineffective when applied topically on skin or mucous membranes and, accordingly, is not administered in that manner. Pharmacokinetic studies with bupivacaine in dogs reveal a longer terminal half-life (30–60 min for IV bolus or brief infusions), although much longer half-lives have been reported following prolonged IV infusions or epidural administration. While bupivacaine elimination is slower than lidocaine and is markedly influenced by dose and route of administration, the prolonged duration of sensory blockade by bupivacaine is substantially longer than would be predicted from differences in the pharmacokinetic profiles of the two drugs. The basis for this prolonged action in some species, as well as a reported proclivity to block sensory nerves longer or more effectively than motor nerves, has led to significant research into the potential basis for these effects.

In contrast to the aforementioned advantages of bupivacaine, the most significant drawback to its use is a heightened risk of acute cardiovascular toxicity, which is cited as a significant concern in human patients. In small animal patients, CVS signs are manifest clinically as moderate to severe ventricular arrhythmias and myocardial depression, especially following inadvertent intravascular administration. Although both lidocaine and bupivacaine rapidly block cardiac Na^+ channels during systole, bupivacaine dissociates from channel binding sites at a slower rate during diastole as compared to lidocaine. This kinetic difference in drug binding is likely to give rise to prolonged blockade of a higher fraction of myocardial Na^+ channels at the end of diastole (Clarkson and Hondeghem, 1985). Thus, the block by bupivacaine is cumulative and substantially greater than would be predicted based upon drug concentration and its local anesthetic potency. At least some component of the adverse cardiac effects of bupivacaine appear to be mediated via actions within the CNS, since direct intracerebral injection of bupivacaine in the medulla can evoke malignant ventricular arrhythmias (Thomas et al., 1986). The clinical management of bupivacaine-induced cardiac toxicity can be very challenging, especially in patients that suffer coexisting respiratory changes with associated acidosis, hypercarbia, and hypoxemia. In addition to these adverse cardiovascular signs, racemic bupivacaine displays some propensity to elicit adverse CNS signs that are similar to signs produced by lidocaine. However, unlike lidocaine, the use of diazepam for treatment of seizures associated with bupivacaine toxicity has been questioned, since both drugs compete for mutual plasma protein binding sites, which could elevate plasma concentrations of free or unbound drug (Moore et al. 1979; Thomas et al., 1976). As discussed above, the most effective means to lessen risk of toxicity with any of the local anesthetics is to limit the total cumulative drug dose that is given to a patient. In this regard, upper dose limits recommended for bupivacaine in dogs (2–3 mg/kg) are lower than the recommended limits for lidocaine (6–10 mg/kg). Recommended limits for both drugs are reduced by 25–50% for cats.

Levobupivacaine

Levobupivacaine is the purified S(-) stereoisomer of racemic (R(+)/S(-)) bupivacaine that was developed, tested, and ultimately marketed and sold in an attempt to diminish the risk of CVS toxicity that had been observed in human patients treated with products containing the racemic mixture. In numerous experimental models involving a range of animal species, levobupivacaine has been found to be less toxic than racemic bupivacaine by as much as 30–60% (see review by Foster and Markham, 2000). Several lines of evidence point to a reduced risk of cardiotoxicity for levobupivacaine compared with R(+)-(*dextro)*-bupivacaine or racemic bupivacaine, including reduced overall effects or lower potencies with respect to blockade of cardiac sodium channels in the inactivated state, blockade of cardiac potassium channels, reduction in the maximal rate of cardiomyocyte depolarization, slowing of atrioventricular impulse conduction speed, and lengthening the duration of the QRS interval. In addition to the stereoselective actions of bupivacaine in cardiac tissues, there is evidence that bupivacaine uptake into the CNS may be enantio-selective insofar as intravenous injections of high (arrhythmogenic) doses of dextrobupivacaine in anesthetized rats have been shown to cause more rapid inhibition of neuronal firing in the nucleus tractus solitarius compared to equivalent doses of levobupivacaine (Denson et al., 1992). Other observations are consistent with enantio-selective CNS actions by bupivacaine stereoisomers, including evidence for a greater frequency of apnea and death in lab animals given dextrobupivacaine compared to equivalent doses of levobupivacaine and a significantly lower threshold for convulsions in sheep with the *dextro* isomer compared to the *levo* isomer (85 ± 11 vs. 103 ± 18 mg; Huang et al., 1998). Finally, a study by Gomez de Segura and colleagues (2009) in dogs suggests that levobupivacaine produces a shorter duration of motor neuron blockade compared to racemic bupivacaine.

Other Selected Agents

Mepivacaine

Mepivacaine is an amide-type local anesthetic with potency and toxicity slightly above that of lidocaine. Although it is the only parenterally administered local anesthetic approved by the FDA (horses), it is not used widely in veterinary medicine. Its unique role has been its use in equine limb blocks where mepivacaine's apparent ability to diffuse though tissue surrounding the injection site creates less edema following drug injection (Bishop, 1996).

Ropivacaine

Ropivacaine (Naropin) is a long-acting amide-type local anesthetic that has efficacy and potency nearly as high as bupivacaine and levobupivacaine but has lower CNS and CVS toxicity. The name ropivacaine has been used to refer to both the racemic mixture as well as the pure S(-) enantiomer. Owing to its expense and availability, ropivacaine is not used commonly in veterinary practice aside from selected reports of uses in dogs and horses. The drug has been reported to produce concentration-dependent effects on peripheral blood vessels, with vasoconstriction at low concentrations

(<0.5% w/v) and vasodilation at concentrations greater than 1 w/v% (Cederholm et al. 1992).

Proparacaine

Proparacaine is an ester-type local anesthetic that is one of two agents approved and marketed specifically for ophthalmic use. The sterile solution (0.5% w/v) is pH adjusted in order to minimize corneal irritation. In general, proparacaine has a faster onset and shorter duration of action than tetracaine, although the half-time for action is longer in dogs (45 min) than cats (15 min).

Procaine

Procaine has historical significance as being the first local anesthetic that was synthesized by chemists. Although it has moderate to low potency and little ability to penetrate mucous membranes, procaine provided evidence that it was possible to produce local anesthesia without the high risk of toxicity and abuse potential that occurs with cocaine. It is rapidly metabolized by plasma cholinesterases into PABA and diethyl-amino ethanol, with the former giving rise to allergic reactions in sensitive animals. The major use of procaine is in the combination with penicillin G to form an insoluble salt that provides slow release and sustained absorption of the antimicrobial drug.

Tetracaine

Tetracaine (Amethocaine) is a derivative of procaine that is approximately 100-fold more potent. It is used primarily for spinal anesthesia and topical anesthesia of the cornea. In ophthalmic applications, it is much longer lasting than proparacaine, even though it can be metabolized rapidly by deesterification.

Benzocaine

Benzocaine is a unique local anesthetic insofar as its use is restricted to topical applications. It has the lowest pK of all local anesthetics that are used clinically, which renders the drug completely unionized at physiological pH. Benzocaine is useful in aquatic species since it can be absorbed readily through the fish's gills and produce general anesthesia. At higher doses, anesthesia can progress to death and benzocaine has been used for chemical euthanasia in fishes. An older use of benzocaine was a laryngeal spray formulation to facilitate airway intubation in cats, but that has been discontinued owning to methemoglobinemia caused by the metabolite benzoic acid.

Future Outlook

There are many areas for potential improvements and expansion of the clinical uses for local anesthetic agents, especially with respect to the pharmacological management of chronic pain. As is true for almost any class of therapeutic agents, there is an ongoing search for better and safer drugs with improved properties related to their spectrum of action, diminished side effects, improved safety profile, and duration of action. With regard to the local anesthetics, development of topically applied agents with rapid onset would be a significant advance insofar as such agents could have reduced systemic side effects and potentially be effective for long-term treatment of localized chronic pain. With further refinement of existing drugs or the development of novel agents, topical analgesics could offer relief for chronic pain conditions that are currently challenging to treat, such as pain resulting from burns, wound debridement, and other conditions. With continued advances in targeted drug-delivery systems, topical analgesics may be able to provide a method to prevent or mitigate phenomena that are difficult to manage clinically, including peripheral or central sensitization as well as neuroplastic changes that may be responsible for the transition from acute to chronic pain states in patients. In this regard, there may be opportunities for targeting novel molecular sites that are distinct from neuronal sodium channels. In addition, the rich and diverse array of sodium channels in different classes of nerves and excitable tissues present some potential for identification of agents that exert selective actions on molecular targets in sensory nerves that transmit pain impulses, without affecting sodium channels in other classes of sensory neurons as well as motor neurons. In this regard, the discovery that selected local anesthetics are able to cross nerve membranes and gain access to target sites within sodium channels by passage through a family of cation-selective membrane channels (TRPV-1 channels) offers an avenue for greater functional selectivity by these agents. Since TRPV-1 and other membrane channels are selectively expressed in selected classes of peripheral sensory neurons, there is potential to develop analgesics that utilize this pathway to exert improved selectivity for pain transmission pathways. Beyond that, entirely new molecular targets could be identified for actions of these drugs. As noted in Section Lidocaine, several metabolites of lidocaine have direct influence on transporters for the inhibitory neurotransmitter glycine in the spinal cord. Since it is recognized that inhibitory glycinergic neurotransmission is impaired in chronic pain states, this or related molecular targets may provide an opportunity for new drug development. Inhibitors of the glycine transporter 2 (GlyT2) are known to enhance inhibitory neurotransmission and have shown particular promise for the treatment of neuropathic pain (Vandenberg et al., 2014). These are just a few of the many examples of opportunities for important new developments with local anesthetics and serve to underscore the need for continued research into effective means to treat patients, both human and nonhuman, with treatment-resistant pain.

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16

Euthanizing Agents

Anna Hampton

Introduction

Euthanasia is derived from the Greek terms *eu* (good) and *thanatos* (death) and is considered a method to end an individual's life as rapidly as possible with little to no pain or distress associated with the procedure (American Veterinary Medical Association, 2013; Close et al., 1996, 1997). In order to be considered euthanasia, the procedure must result in rapid loss of consciousness, followed by loss of vital body functions (respiration, heartbeat, and brain function). It is crucial that euthanasia be performed with consideration of the animal's welfare and with the highest degree of respect. In addition, all aspects of euthanasia, not just the method chosen, should be evaluated to ensure that pain and distress are minimized. This includes preeuthanasia procedures such as handling, restraint, and the environment, as well as confirmation of death (Canadian Council On Animal Care in Science, 2010; United Kingdom House of Commons, 1986). Furthermore, it is prudent that the animal's remains and euthanasia solutions are used and disposed of appropriately and legally (American Veterinary Medical Association, 2013).

Euthanasia methods for animals can be divided into two primary groups (chemical and physical methods) and three basic mechanisms (direct depression of neurons vital for life functions, hypoxia, and physical destruction of neurons and brain activity vital for life). This chapter solely focuses on the pharmacology of chemical methods of euthanasia. Regardless of the group, the following items should be considered on a case-by-case basis when choosing a method of euthanasia, as outlined by the AVMA *Guidelines for the Euthanasia of Animals*: *2013 Edition*, and other core documents (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997; United Kingdom House of Commons, 1986):

- ability to induce loss of consciousness and death with a minimum of pain and distress;
- time required to induce loss of consciousness;
- reliability and reproducibility;
- safety of personnel;
- training of personnel for all aspects;
- required restraint and handling;
- ease of administration;
- irreversibility;
- compatibility with species, age, and health status;
- compatibility with intended animal use and purpose;
- compatibility with subsequent evaluations, examinations, or use of tissue;
- drug availability and human abuse potential;
- ability to maintain equipment in proper working order;
- safety for predators or scavengers should the animals remains be consumed;
- legal requirements;
- environmental impacts of the method and disposition of the animal's remains;
- emotional effect on observers or operators.

It is essential that personnel are educated and trained in the appropriate and safe techniques for all aspects of euthanasia, which includes preeuthanasia handling and restraint, and understand the underlying justifications for euthanasia. This not only aides in the humane treatment of animals, ensuring as little distress as possible, but can also help mitigate any psychological impacts on the individual (caring–killing paradox) (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010). In addition, if the method chosen involves controlled substances, it is important to institute proper storage and diversion control to prevent abuse, and in some cases suicide. Most euthanasia agents have a narrow therapeutic index or excessive side effects and are not used in human medicine. Acepromazine, xylazine, T-61, barbiturates, and others have been used in suicide or suicide attempts (Perri, 2015; Giorgi and Bertini, 2000).

It is beyond the scope of this chapter to discuss animal slaughter or killing of wild game for sport.

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Inhaled Agents

In general, inhaled methods of euthanasia fall into one of two mechanisms: (i) hypoxia and/or (ii) direct depression of neurons vital for life functions. It is imperative that crucial concentrations of the gases and vapors in the alveoli and blood are achieved to get the desired effect, rapid unconsciousness. Gas displacement, container volume, and gas concentration can all affect the time to onset of unconsciousness. Gases should be delivered in a calibrated and purified from; this is commonly accomplished through the use of a flow meter and/or pressure-reducing regulator (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010). Inhaled methods of euthanasia are not recommended for animals that are resistant to hypoxia or hold their breath like poikilotherms, diving animals, and neonatal animals (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997; Pritchett et al., 2005; Pritchett-Corning, 2009). In addition, rabbits tend to be distress when exposed to gasses and other methods may be more appropriate for this species (Close et al., 1996, 1997). Although it can be difficult to determine if gas exposure is distressful to a specific species or individual, behavior while being exposed to the agent or aversion testing can provided some information. The results, as discussed in this section, can produce a large breath of recommendations for specific species or situations when using inhaled agents, which highlights the importance of choosing a method for specific situations and species. As a general recommendation, death should be confirmed by cession of vital signs or a definitive, secondary method of euthanasia should be performed to prevent recovery.

Carbon Dioxide

Inhaled carbon dioxide is likely one of the most studied and disputed euthanasia methods available. Because it is rapid, safe, inexpensive, and readily available it is the most common method of euthanasia for laboratory rodents (Artwohl et al., 2006). Respiratory exposure to CO_2 causes respiratory acidosis, which in turn creates a physiological domino effect. Acutely, this small molecule rapidly diffuses from the lungs into the plasma and intracellular spaces, and when combined with water it produces carbonic acid which rapidly splits into bicarbonate and hydrogen ions. If the buffering capacity is exceeded in this dose-dependent process you get respiratory acidosis. The resulting acidification of the intracellular space and cerebrospinal fluid leads to suppressed nerve cell function and inhibition of central *N*-methyl-D-aspartate (NMDA) receptors causing anesthesia (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Martoft et al., 2003; Pritchett et al., 2005; Thomas et al., 2012). Prolonged exposure to CO_2 and/or high concentrations of CO_2 then leads to severe respiratory acidosis and depression of the respiratory centers of the brain, eventually resulting in death. Other actions include acidosis-induced depression myocardial contractility and direct myocardium effects (American Veterinary Medical Association, 2013; Pritchett et al., 2005; United Kingdom House of Commons, 1986). Although eventual hypoxia results, it is not the direct mechanism of action resulting in death.

The ideal rate of CO_2 delivery appears to be species specific. For rodents, there has been conflicting reports with some recommending use of a prefilled chamber (100% CO_2) for euthanasia (Artwohl et al., 2006; Canadian Council On Animal Care in Science, 2010), while others recommend a gradual fill method, with a fill rate of approximately 10–20% of chamber volume per minute (American Veterinary Medical Association, 2013; Burkholder et al., 2010; Close et al., 1996, 1997; Conlee et al., 2005). Conflicting recommendations have also been published for swine and poultry (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Grandin, 1994). Furthermore, 100% exposure is recommended in rabbits and ferrets, but these animals may also be distressed at any exposure (Close et al., 1996, 1997; Fitzhugh et al., 2008). Regardless of the rate of delivery, use of compressed CO_2 gas in cylinders with flowmeters to precisely deliver the gas is the only acceptable source (American Veterinary Medical Association, 2013; Artwohl et al., 2006; Canadian Council On Animal Care in Science, 2010; United Kingdom House of Commons, 1986). Euthanasia can be accomplished over a wide range of concentrations (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997; Leach et al., 2002; United Kingdom House of Commons, 1986).

Many studies have looked at effect of gas combinations to mitigate the negative aspects of CO_2 euthanasia, with variable results. It has been suggested that the addition of nitrous oxide to CO_2 decreases the time to unconsciousness in mice and pigs, thus likely decreasing the distress the animal experiences (Llonch et al., 2012; Rault et al., 2013; Thomas et al., 2012).

Respiratory acidosis and other physiological events that occur during exposure to CO_2 leads to successful euthanasia, but may also play a role in potential pain and distress an animal experiences when exposed to CO_2. Resulting lactic acidosis leads to activation of the acid sensing ion channels (ASIC), which may cause pain or fear (American Veterinary Medical Association, 2013; Thomas et al., 2012). In addition, as indicated earlier, carbonic acid is created when CO_2 combines with water. This reaction occurs in the ocular, nasal, and oral mucous membranes and carbonic acid activates polymodal nociceptors and has an irritating effect on these areas (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Hawkins et al., 2006; Leach et al., 2002; United Kingdom House of Commons, 1986). CO_2 also has a pungent odor and hypoxia may lead to a feeling of breathlessness, thus possible distress if unconsciousness is not rapid (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Hawkins et al., 2006; Leach et al., 2002). Because of carbonic acid production, CO_2 should not be dissolved in water to euthanize aquatic species (Canadian Council On Animal Care in Science, 2010). Some recommend that it not be used as a sole agent in species that have shown significant aversion to CO_2 exposure such as pigs, mink, ferrets, and rodents (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010; Conlee et al., 2005; Fitzhugh et al., 2008; Leach et al., 2002; Makowska et al., 2009; Valentine et al., 2012). However, other studies have shown that it can be used successfully in pigs and rodents (Burkholder et al., 2010; Grandin, 1994; Hackbarth et al., 2000; Sharp et al., 2006).

Since CO_2 exposure is the most common method of euthanasia for laboratory rodents, it is important to consider the biochemical effects and possible artifacts that may be created if this method of euthanasia is elected. CO_2 exposure can stimulate enzymes in the glycolytic pathway, and affect red blood cell parameters (Artwohl et al., 2006). In addition, lung pathological artifacts such as edema and hemorrhage have been reported (Danneman et al., 1997; Fawell et al., 1972).

Advantages of CO_2 as a euthanasia agent is that it is heavier than air so less likely to displace out of container (however, consider the height of the animal), generally has a rapid onset, is relatively inexpensive, and nonflammable and nonexplosive (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Llonch et al., 2012). However, the advantages must be heavily weighed with the substantial and conflicting reports on the potential for pain and distress with this method, which make it difficult to have generalized recommendations.

Carbon Monoxide

Carbon Monoxide (CO) is a colorless, odorless gas that is produced by the chemical interaction of sodium formate and sulfuric acid. It preferentially combines with the iron in hemoglobin producing carboxyhemoglobin, blocking uptake of oxygen and inhibiting other binding sites to off-load oxygen, resulting in hypoxia (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Makowska and Weary, 2009). This leads to loss of consciousness, eventually resulting in death. During loss of consciousness the motor centers of the brain are stimulated, thus this may be accompanied by convulsions and muscle spasms. In general CO produces rapid loss of consciousness and death with minimal pain and distress at low concentrations (4–6%), although this may be species dependent (American Veterinary Medical Association, 2013; Makowska and Weary, 2009). Use of a presedative may decrease the signs of distress in dogs (Dallaire and Chalifoux, 1985). The main concern with use of CO for euthanasia is the occupational risk involved to personnel. It is a human hazard and can be explosive in high concentrations. The environment should be equipped with an effective exhaust or ventilation system, CO monitors, and spark free and explosion-proof electrical equipment (American Veterinary Medical Association, 2013; Close et al., 1996, 1997). Only commercial cylinders should be used for euthanasia (Close et al., 1996, 1997).

Argon

Argon (Ar) is an odorless, colorless, and tasteless inert gas. It is nonflammable and nonexplosive. Its mechanism of action, producing unconsciousness and eventual death, is achieved by displacing air/oxygen causing anoxia. Production of unconsciousness is rapid if oxygen levels are <2% and gradual exposures are not recommended as they result in prolonged exposure to hypoxic conditions prior to unconsciousness, and resulting distress. It is important that death is confirmed before oxygen levels are reestablished to prevent recovery (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010). In general, euthanasia with Ar requires longer exposure times when compared to CO_2 and its use has been described in swine, poultry, and rodents. This being said, exposure response and aversion appears to be species dependent and anesthesia prior to exposure has been recommended (American Veterinary Medical Association, 2013; Burkholder et al., 2010; Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997; Leach et al., 2002; Llonch et al., 2012; Makowska et al., 2009; Sharp et al., 2006).

Nitrogen

Nitrogen has very similar properties to argon, described in Section Argon. It produces death by the same mechanism as argon, displacement of air and subsequent anoxia with unconsciousness being reached if O_2 is <2%. It too has been described as a euthanasia agent in poultry, swine, and rodents, with species variability (American Veterinary Medical Association, 2013; Close et al., 1996, 1997; Llonch et al., 2012; Sharp et al., 2006). An important application of nitrogen asphyxiation is for mass emergency depopulation of commercial poultry operations (Benson et al., 2007; National Turkey Federation, 2013). This approach uses medium expansion water-based firefighting foams (nitrogen, water, foam concentrate). This has recently been applied in depopulation of flocks exposed to highly pathogenic avian influenza (HPVI).

Inhalant Anesthetic Overdose

An anesthetic is an agent that produces drug-induced depression of the nervous system, unconsciousness, analgesia, muscle relaxation, and absence of perception of all sensation, in a controllable manner. When an anesthetic overdose is given, it results in eventual cessation of vital signs resulting in death (Close et al., 1996, 1997; Makowska et al., 2009). Typically, this is accomplished by blocking interactions of specialized brain regions or decreasing the activity patterns in the brain, with an overall decrease of input to the cerebral cortex and depression of the cardiovascular and respiratory system (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010). Supplying an overdose of an anesthetic agent is generally effective, if the anesthetic is appropriate for that species; however, the time to death can be lengthy and supplemental oxygen should be provided (Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997). There are numerous gases that can be used for anesthesia; currently only isoflurane, enflurane, sevoflurane, and desflurane are clinical available in the United States, but clinicians may be able to obtain halothane and methoxyflurane form other suppliers. Isoflurane may artificially elevate blood glucose levels. Enflurane and sevoflurane cause electrocortical activity, behaviorally resulting in convulsions under anesthesia. The order of preference outlined in the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* are: isoflurane, halothane, sevoflurane, enflurane, methoxyflurane, and desflurane, with or without nitrous oxide (ether is not acceptable) (American Veterinary Medical Association, 2013).

Injected Agents

Injectable agents delivered parenterally are generally the most rapid, reliable, and preferred method of euthanasia for most species. However, it is important to remember they typically require more handling and restraint, which may be a stressful event for the animal and pose an occupational hazard for the individuals(s) involved (American Veterinary Medical Association, 2013). Intravenous administration is the preferred route for most injectable agents, but in small or debilitated animals, where vascular access can be difficult, other routes can be considered but may have drawbacks (Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997; United Kingdom House of Commons, 1986). As a rule of thumb, three to four times the therapeutic dose of an anesthetic will produce rapid and uniform cardiac arrest and death (Close et al., 1996, 1997).

Barbiturate (Pentobarbital)

Barbiturates are the most widely used and accepted agent for euthanasia, reliably resulting in a smooth, rapid euthanasia. Sodium pentobarbital is generally the most suitable, but this group also includes barbituric acid derivatives, oxybarbiturates (sodium pentobarbitone, secobarbital), thiobarbiturates (thiopentone), and barbiturate mixtures (American Veterinary Medical Association, 2013; Close et al., 1996, 1997). Barbiturates cause severe depression of the central nervous system. They interfere with sodium and potassium transport across cell membranes leading to inhibition of the mesencephalic reticular activation system, blocking postsynaptic transmission in all areas of the central nervous system. In addition, they act as positive allosteric modulators and at high doses they bind directly to the γ-amiobutyric acid (GABA) A receptor activating it. This induces a dose-dependent rapid sedation, hypnosis, anesthesia, and eventually respiratory depression and death (Clark et al., 2012; Close et al., 1996, 1997; Perrin, 2015).

Postmortem barbiturate overdose may produce blood parameter changes and pathological artifact both systemically and locally, including congestion, edema, hemorrhage, hemolysis, emphysema, and necrosis (Artwohl et al., 2006; Grieves et al., 2008).

Proper disposal of both the agent, as a controlled substance, and carcass, to avoid poisoning of scavenging wildlife, is important (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010). The advantages of its use, for most species, far outweigh the disadvantages. In addition, there are many pentobarbital combination agents available that include a mixture of a barbiturate and a local anesthetic, other central nervous system depressant, or agents that enhance the metabolism of the barbiturate. This increases the toxicity, but the agents are specifically made for euthanasia and have no therapeutic use (American Veterinary Medical Association, 2013).

Tributame

Tributame is a nonbarbiturate euthanasia agent that contains embutramide, chloroquine phosphate, and lidocaine, which is FDA approved for euthanasia in dogs. Embutramide, a derivative of γ-hydroxybutyrate, is an anesthetic with no therapeutic value due to its severe cardiovascular effects, which include hypotension, myocardial depression, and ventricular dysrhythmias resulting in death. Chloroquine phosphate, an antimalarial drug, was added to shorten the time to unconsciousness and death as it is a profound cardiovascular depressant. The local anesthetic lidocaine was added to reduce the pain associated with intravenous injection in cats. Death occurs as a result of central nervous system depression, respiratory depression and hypoxia, and circulatory collapse. It should only be administered intravenously and caution should be taken if chosen as a euthanasia agent for cats as agonal breathing may occur (American Veterinary Medical Association, 2013).

T-61 (Embutramide, Mebozonium Iodine, and Tetracaine Hydrochloride)

T-61 (Tanax®) is a nonbarbiturate, nonnarcotic mixture of embutramide, mebozonium (mebezonium) iodine, and tetracaine hydrochloride. Embutramide is a general anesthetic that has a strong narcotic effect and acts by causing respiratory depression in the central nervous system and induces deep anesthesia. Mebenzonium iodide is a nondepolarizing neuromuscular blocking agent that paralyzes the skeletal muscle and rapidly induces respiratory collapse. Lastly, T-61 contains the powerful, local anesthetic, tetracaine hydrochloride, which reduces pain at the injection site (American Veterinary Medical Association, 2013; Giorgi and Bertini, 2000; Hellebrekers et al., 1990; Raghav et al., 2011). It is recommended that animal are sedated prior to administration and that it is given slowly, intravenously. It has been described as a euthanasia agent for dogs, amphibians (dorsal lymph sac), small birds (intramuscular), and mammals (intravenous) (Close et al., 1996, 1997). In general, it has a similar induction of euthanasia as pentobarbital; however, you may not see the terminal gasp that sometimes accompanies barbiturate overdose. However, convulsions in birds have been reported (Giorgi and Bertini, 2000; Hellebrekers et al., 1990). There has been concern that an animal might experience paralysis of the diaphragm prior to loss of consciousness, but this has been unfounded with a study showing that loss of consciousness and loss of muscular activity occur simultaneously in dogs and rabbits (Hellebrekers et al., 1990). Postmortem tissue lesions, including endothelial necrosis, hemolysis, pulmonary congestion, and edema, have been described (Giorgi and Bertini, 2000; Raghav et al., 2011). T-61 is no longer marked in the United States but may be available elsewhere (American Veterinary Medical Association, 2013).

Other Injectable Anesthetics and Sedatives

Etorphine hydrochloride and carfentanil citrate are ultrapotent opioids that act on the μ opioid receptors to cause profound CNS and respiratory depression, which leads to death. They are FDA approved for wildlife or exotic animal immobilization and at high enough doses can be used for euthanasia. Caution should be used given the human risk of handling the drug (American Veterinary Medical Association, 2013). Although it is generally not recommend administering euthanasia agents orally due to their prolonged onset of action, inaccurate dosing, and possibility of tissue irritation, carfentanil citrate is absorbed transmucosally and has been proposed as a euthanasia method for great apes when delivered in lollipops (Close et al., 1996, 1997; Kearns et al., 1999, 2000).

The combination of a dissociative agent and an α-adrenergic receptor agonist has been used successfully for euthanasia. The most common duo used in laboratory rodents is an overdose of ketamine (a dissociative agent and NMDA-receptor antagonist) and xylazine (α-adrenergic receptor agonist). Together they induce rapid loss of consciousness and death. The preferred route of administration is intravascular; however, it can be given intraperitoneal, intramuscular, or retroorbital (American Veterinary Medical Association, 2013; Schoell et al., 2009). Given the small muscle mass in mice, intramuscular is not recommended. In addition, an overdose of the injectable hypnotic agent, propofol, may be considered for euthanasia if deemed acceptable for the species and environment (American Veterinary Medical Association, 2013).

Potassium Chloride

Potassium chloride is a cardiotoxin, which at high levels delays electrical conduction between myocytes, resulting in sinoatrial and atrioventricular block, and thus cessation in ventricular conduction. This leads to ventricular fibrillation and fatal asystole. The induced cardiac arrest can generate convulsive seizures, muscle spasms, gasping, and vocalization (Close et al., 1996, 1997; Raghav et al. 2011). Given that the mechanism of action is induction of cardiac arrest without prior unconsciousness, potassium chloride can only be used after the animal is under an surgical plane of anesthesia or rendered unconscious (American Veterinary Medical Association, 2013).

Immersion Agents

General

Immersion methods of euthanasia are generally recommended for aquatic species (e.g., amphibians and fish). Depending on the species it may be absorbed through the gills, ingested, and/or through the skin. It is important that introduction into the dissolved euthanasia agent does not induce pain or distress, thus the pH, temperature, and irritating potential of the solution should be considered. In addition, the water quality, especially temperature, may alter the efficacy of the drug and induction of euthanasia (American Veterinary Medical Association, 2013; Close et al., 1996, 1997). Given that aquatic animals generally have slow metabolic rates and it can be difficult to determine cessation of vital signs, a definitive secondary physical or chemical method is recommended to ensure nonrevival (Canadian Council On Animal Care in Science, 2010). Of note, the *AVMA Guidelines for the Euthanasia of Animals: 2013 Edition* describes immersion in CO_2-saturated water as an acceptable method of euthanasia; however, the Canadian Council on Animal Care guidelines does not find it acceptable and there have been other reports that it does not meet definition of euthanasia, thus it should be used only with great consideration (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010; Matthews and Varga, 2012; Neiffer and Stamper, 2009).

Tricaine Methane Sulfonate (MS-222)

Tricaine methane sulfonate, also known as MS-222, is a benzoic acid derivative commonly used as an anesthetic and euthanasia agent in fish and amphibians. It causes direct depression of the central nervous system by altering the properties of voltage-gated sodium channels, thus blocking the generation of action potentials. At doses five to ten times the anesthetic dose it results in depression of the respiratory system, and accumulation in the myocardium results in decreased cardiovascular function, ultimately resulting in death (American Veterinary Medical Association, 2013; Neiffer and Stamper, 2009; Torreilles et al., 2009). Soluble in both salt and fresh water, the MS-222 must be buffered with sodium bicarbonate prior to placement of the animal in the solution to reduce irritation and skin damage. Like most immersion agents, its efficacy is dependent on the species, animal size, and water quality. For example, an increase in water temperature increases potency (Close et al., 1996, 1997). The solution can be poured over the gills of fish that are too large to immerse (Neiffer and Stamper, 2009).

Benzocaine Hydrochloride

Used as a euthanasia agent in finfish and amphibians, benzocaine hydrochloride has a similar mechanism of action to MS-222. It blocks voltage-sensitive sodium channels within the central nervous system, resulting in depression of the nervous, respiratory, and cardiovascular system, eventually resulting in death (American Veterinary Medical Association, 2013; Torreilles et al., 2009). Benzocaine hydrochloride is not water soluble and must be dissolved in acetone before adding to water. In solution, it will decrease the pH of the tank and caution should be taken to ensure the water is not too acidic before immersing the animal.

2-Phenoxyethanol

Immersion in 2-phenoxyethanol is considered acceptable for the euthanasia of finfish. The mechanism of action is poorly understood but is thought to be secondary to central nervous system depression. Some species variation may be seen with regard to dose and time to loss of consciousness. In addition, some species may show hyperactivity prior to unconsciousness (American Veterinary Medical Association, 2013).

Quinaldine Sulfate

Quinaldine sulfate is a one-step, immersion euthanasia method for finfish. It accumulates in lipid tissue and causes depression of the sensory centers of the central nervous system. The dose to achieve euthanasia is dependent on species, and water quality (i.e., temperature, pH, and mineral content) and induction may be prolonged. It is not water soluble and must be dissolved in acetone prior to making a tank solution (American Veterinary Medical Association, 2013; Close et al., 1996, 1997).

Other Anesthetic Solutions

In general, an overdose of an anesthetic solution will result in death. Cloves contain essential oils (e.g., isoeugenol, eugenol, and methyleugenol) and when used at high concentrations rapidly cause ventricular failure in finfish. Again, the mechanism of action is poorly understood but, similar to other immersion anesthetic agents, it likely inhibits voltage-sensitive sodium channels within the central nervous system (American Veterinary Medical Association, 2013). Inhalant anesthetics (halothane, isoflurane, servoflurane, CO_2) can be bubbled through the tank; however, given the wide range of species variation, this should be considered on an individual basis (American Veterinary Medical Association, 2013; Canadian Council On Animal Care in Science, 2010; Close et al., 1996, 1997).

Miscellaneous Considerations

Topical Benzocaine Gel

Like oral administration, topical absorption of a euthanasia agent is generally slow and efficacy is variable, thus in general this route is generally unacceptable. However, it can be used successfully in animals with highly permeable skin (i.e., amphibians). Benzocaine gel can be placed on the skin of amphibians (American Veterinary Medical Association, 2013; Torreilles et al., 2009).

Sodium Hypochlorite

Sodium hypochlorite (bleach) can be used to euthanize zebrafish up to 7 days postfertilization, prior to nociception. It causes saponification of fatty acids, denatures proteins, and induces derangement of the cellular process ceasing development (American Veterinary Medical Association, 2013).

Invertebrates

Until this paragraph, this chapter has focused on agents used to euthanasia vertebrate animals. However, more information is becoming available on appropriate agents for the euthanasia of invertebrates. The *AVMA Guidelines for the Euthanasia of Animals*: *2013 Edition*, mentions the use of alcohol or formaldehyde in anesthetized aquatic invertebrates, immersion in anesthetic solutions (i.e., magnesium salts, clove oil, eugenol) for aquatic invertebrates, and inhaled anesthetic overdoses or injectable agents for terrestrial invertebrates (American Veterinary Medical Association, 2013).

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17

Anticonvulsant Drugs

Mark G. Papich

Introduction

Treatment of seizure disorders first requires that an accurate diagnosis is made and underlying problems ruled out, such as metabolic disorders, neoplasia, congenital disorders, and intoxication. If a diagnosis of idiopathic epilepsy is made, treatment is usually initiated with one of the maintenance anticonvulsants described in this chapter. Fortunately, excellent current guidelines are available from experts that help guide drug selection (Podell et al., 2016; Bhatti et al., 2015). An evidence-based analysis of anticonvulsant studies in animals provides veterinarians information to make decisions about selection of drugs (Charalambous et al., 2014). Occasionally, animals are presented to veterinary hospitals with continuous seizures – status epilepticus. These animals require prompt treatment with one of the rapidly acting anticonvulsants.

The methods used to classify and diagnosis seizure disorders will not be discussed in this chapter. One should rely on a reputable internal medicine textbook, review paper, or reference that specifically addresses seizure disorders in animals (e.g., Podell, 2013; Muñana, 2013).

In recent Task Force and specialty Consensus Statement Reports (Podell et al., 2016; Bhatti et al., 2015) these drugs are referred to as *antiepileptic drugs* (AED). Here, we will use the term anticonvulsant drugs, which for the purpose of this chapter will be considered interchangeable terms. Anticonvulsant drugs act to limit either the initialization or spread of the seizure focus in the central nervous system. These drugs act to suppress nerve conduction, stabilize neurons, or enhance and potentiate the action of the inhibitory neurotransmitter γ-aminobutyric acid (GABA). For a few drugs, the exact mechanism of action is still unknown. Some drugs, such as the barbiturates, are used for other indications as well. Some barbiturates are better at providing an anticonvulsant effect, and others are better at producing anesthesia. An anticonvulsant is distinguished from an anesthetic by an action that suppresses seizure activity, without producing unconsciousness. Table 17.1 lists anticonvulsant drugs used in veterinary medicine. Some are no longer in use, or are rarely used.

The application of pharmacokinetic principles to treatment of epilepsy in animals has been a major contribution to veterinary neurology. Therefore, the focus of many research publications has been on the pharmacokinetics of the anticonvulsant medications. The pharmacokinetics of several anticonvulsants in dogs, with comparison to humans, was reported by Frey and Löscher (1985), and other papers over the years have followed this pattern with the intent of examining the pharmacokinetics of human-labeled drugs in animals for the purpose of evaluating their potential for veterinary anticonvulsant use. Other relevant papers will be discussed in this chapter among individual drugs. In the paper by Frey and Löscher (1985), they reported significant differences for some drugs, with generally much more rapid clearance in dogs than humans. Protein binding was generally similar for drugs in dogs and people, with the exception of valproate and phenytoin.

The most recent consensus papers and task force reports by experts on this topic (Podell et al., 2016; Potschka et al., 2015; Bhatti et al., 2015) have helped tremendously to guide veterinarians on the best drugs to consider for initial use, the dosages, adverse effects, and evidence to support the clinical use. Drugs most often listed in these guidelines for seizure management include: phenobarbital, bromide, primidone, imepitoin, levetiracetam, and zonisamide. Imepitoin is not approved in the United States, but is available in other countries.

Phenobarbital

Phenobarbital is the most widely used anticonvulsant drug in small animals. Although it is classified as a barbiturate (Figure 17.1) and shares properties with barbiturates discussed in Chapter 12, phenobarbital is unique in its ability to produce an anticonvulsant effect at doses below those that produce anesthesia.

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Table 17.1 Anticonvulsants that have been used in veterinary medicine

Barbiturates	Phenobarbital Mephobarbital Primidone
Hydantoins	Phenytoin
Succinimides	Ethosuximide
Benzodiazepines	Diazepam Clonazepam Oxazepam Midazolam Lorazepam
Oxazoldinediones	Trimethadione Paramethadione
Others	Imepitoin Valproate Carbamazepine Potassium bromide Levetiracetam Gabapentin Pregabalin Zonisamide

Mechanism of Action

Phenobarbital increases the seizure threshold and decreases the electrical activity of the seizure focus by potentiating the action of the neurotransmitter GABA. Potentiating the $GABA_A$ subunit increases chloride conductance in neurons, stabilizes electrical activity, and raises the potential necessary for depolarization. Barbiturates also decrease the influx of calcium into nerve cells and thereby decrease release of neurotransmitters. This property may be more important at high phenobarbital levels than at the concentrations that produce an anticonvulsant effect.

Pharmacokinetics

The pharmacokinetics of phenobarbital have been studied in a variety of animals (Frey and Löscher, 1985). Protein binding in serum is 24–30% in sheep, 34–52% in goats, 31% in horses, 19–28% in rabbits, 10–25% in pigs, and 4–23% in cows (Bailey, 1992). This compares with a value of 30–38% in humans.

Phenobarbital is well absorbed with systemic availability that is almost complete in monogastric animals (Pedersoli et al., 1987). The volume of distribution is large and is similar to total body water at 600–700 ml/kg. The high lipophilicity produces penetration across lipid membranes, including the blood–brain barrier of the central nervous system (CNS). Phenobarbital clearance is low in dogs (0.09–0.1 ml/kg/min) (Pedersoli et al., 1987), but it is highly metabolized and relies on hepatic cytochrome P450 enzymes for biotransformation. In people the half-life is in the range of 70–100 hours. In dogs the range has been from 37 to 75 hours (average 53 hours) (Ravis et al., 1984), 89 ± 20 hours (Ravis et al., 1989), and 92.6 (± 24) hours (Pedersoli et al., 1987). In a study that examined variations during the dosing interval in epileptics, the range in half-life among dogs was 20–140 hours, with a mean of 65 hours

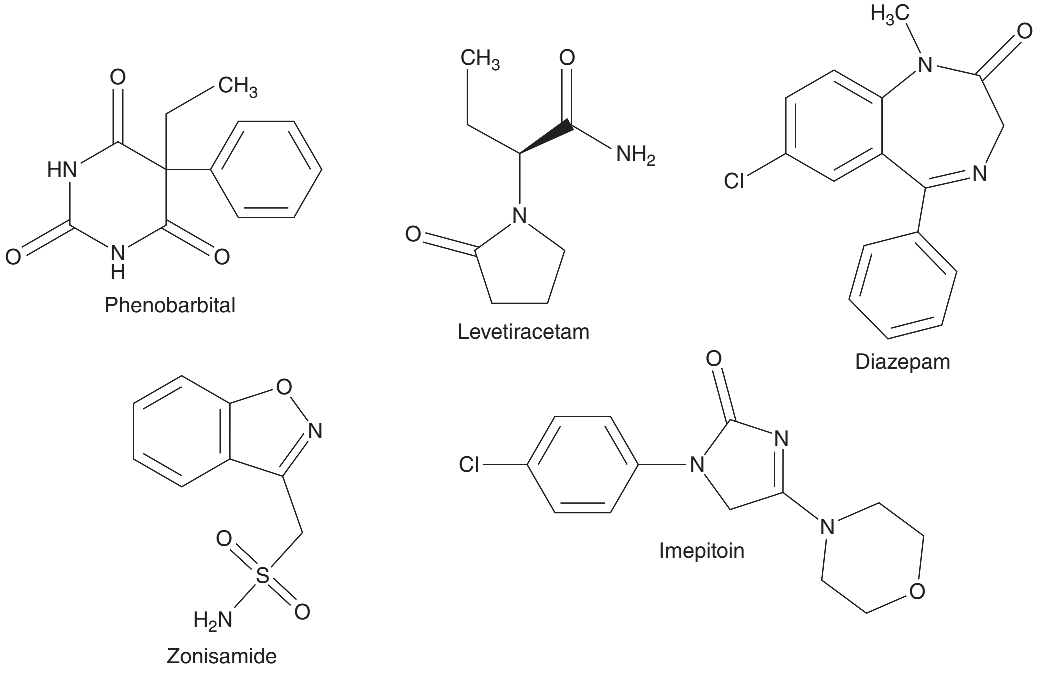

Figure 17.1 Structure of common anticonvulsant drugs used in veterinary medicine.

(Levitski and Trepanier, 2000). As demonstrated from these studies, the half-lives can vary considerably among animals, which may explain a range of plasma concentrations despite similar doses among dogs. This degree of variability supports individualized dosing based on therapeutic drug monitoring. Changes in half-life and clearance that occur during multiple dosing is caused by autoinduction of hepatic metabolism (Ravis et al., 1989; Hojo et al., 2002), and may also be influenced by diet (Maguire et al., 2000). With multiple dosing, the half-life decreased from 89 hours (± 19.6) on day 1 to 47 hours (± 11) at day 90. After multiple dosings produce autoinduction of hepatic enzymes, clearance almost doubled in dogs (Ravis et al., 1989). After multiple oral treatments with phenobarbital, the liver cytochrome P450 content was twofold higher and plasma concentrations lower, compared to untreated dogs (Hojo et al., 2002). The activity of CYP1A, 2C, and 3A were increased by two- to fourfold compared to untreated dogs.

In cats the half-life ranges from 35 to 56 hours (average 43 hours) and from 59 to 76 hours, depending on the study (Cochrane et al., 1990a, 1990b). In horses the half-life is approximately 18–19 hours; however, one study showed a half-life of 24 hours after a single dose but 11.2 hours after multiple doses (Knox et al., 1992; Duran et al., 1987; Ravis et al., 1987). In foals the mean half-life was 13 hours (Spehar et al., 1984). These values for horses indicate a more rapid clearance in horses than in other species. In birds the half-life is short, which makes it difficult to maintain effective concentrations. Despite high doses in parrots, the half-life was only 1.4–1.7 hours, with low peak concentrations (Powers and Papich, 2011).

Drug Interactions

Phenobarbital is a well-known inducer of microsomal cytochrome P450 (CYP450) enzymes (microsomal enzymes) that metabolize drugs (Hojo et al., 2002). Phenobarbital is an inducer of canine CYP2B11, CYP3A12, CYP2C21/2C41, and possibly others (Martinez et al., 2013). Increased amounts of CYP450 enzymes have been documented and are found in the endoplasmic reticulum of hepatocytes.

Induction of microsomal enzymes may cause enhanced biotransformation of other drugs, which can diminish the pharmacological effect for a drug that is administered concurrently (e.g., digoxin, corticosteroids, phenylbutazone, some anesthetics, and antipyrine). When phenobarbital was administered with another anticonvulsant to dogs, levetiracetam, the clearance and half-life of levetiracetam was significantly altered, producing a shorter half-life and faster clearance in dogs (Moore et al., 2011). The authors of the study concluded that concurrent phenobarbital administration significantly alters the pharmacokinetics of levetiracetam in dogs, indicating that dosage adjustments might be necessary when the drug is administered with phenobarbital. Phenobarbital will also increase the clearance of zonisamide by at least 50%, requiring an adjustment of the dose of zonisamide if it is administered with phenobarbital (Orito et al., 2008). (Zonisamide and levetiracetam are discussed later in this chapter.) It was also observed that if a canine patient has been receiving maintenance therapy with phenobarbital, the concentration of diazepam and metabolites were lower after intravenous and rectal administration compared to diazepam administration prior to phenobarbital treatment. Therefore, it may require higher doses of diazepam in some dogs to control periodic seizures because of increased clearance caused by enzyme induction (Wagner et al., 1998). Enzyme induction will also increase phenobarbital's own metabolism. In one study (Maguire et al., 2000), the half-life at the beginning of the study was 47–49 hours in dogs. But, after chronic treatment for 2 months, the phenobarbital half-life decreased to 24–33 hours. In another study, autoinduction of enzymes significantly lowered the phenobarbital plasma drug concentration in dogs compared to the expected level that would ordinarily occur from multiple dosing (Hojo et al., 2002).

Other drugs that are microsomal enzyme inhibitors (CYP450 inhibitors) may inhibit the metabolism of phenobarbital and cause toxicity if the two drugs are administered concurrently. Chloramphenicol and ketoconazole are examples of these CYP450 enzyme inhibitors (Martinez et al., 2013).

Side Effects, Adverse Effects, and Tolerance

Side effects that one may expect include sedation, polyphagia (with associated weight gain), polyuria/ polydipsia, and mild behavior changes. These may subside somewhat after the first few weeks of treatment because both pharmacokinetic and pharmacodynamic tolerance develops with chronic therapy. Pharmacokinetic tolerance is caused by enhanced metabolism owing to induction of the hepatic enzymes (i.e., phenobarbital increases its own metabolism) with chronic dosing that was discussed above. Pharmacodynamic tolerance is determined by the sensitivity of receptors.

Liver: Elevated hepatic enzymes have been observed after chronic therapy in healthy animals (ALT, alanine aminotransferase; ALP, alkaline phosphatase) without an association with hepatic injury (Aitken et al., 2003; Gieger et al., 2000; Müller et al., 2000a; Chauvet et al., 1995). The highest elevations occur with serum ALP. Elevations in hepatic enzymes that are not associated with liver pathology are reported to be caused by induction of the enzymes by the drug and are expected to return to normal 3–5 weeks after the drug is discontinued (Gieger et al., 2000). In one study of 95 epileptic dogs controlled with phenobarbital, the increases in liver enzymes were related to the phenobarbital plasma concentration (Aitken et al., 2003). Dogs with the highest levels of phenobarbital also had the highest ALT increases.

Hepatotoxicosis also is possible from phenobarbital. It has been described as both an intrinsic and idiosyncratic hepatic injury. In clinical reports, most affected dogs had high serum phenobarbital concentrations (>40 μg/ml) (Dayrell-Hart et al., 1991). Animals with phenobarbital-induced hepatotoxicosis had bilirubin increases that were disproportionately elevated in comparison to the ALP. There also may be elevations in bile acids or other signs of hepatic disease. Liver lesions include chronic fibrosis with nodular regeneration, biliary hyperplasia, necrosis, and cirrhosis (March et al., 2004). Animals receiving high doses of phenobarbital to control seizures should be examined periodically because they may be at a higher risk of developing liver disease. Although hepatic injury has been an important concern associated with administration of phenobarbital to dogs, this has not been a documented problem in cats.

Hepatocutaneous disease: As a consequence of phenobarbital-induced liver disease, the skin also may be affected. This has been described as *superficial necrolytic dermatitis,* more commonly known as *hepatocutaneous syndrome*. The hepatocutaneous syndrome has been associated with phenobarbital administration in some dogs (March et al., 2004). Prolonged phenobarbital exposure and high plasma drug levels may contribute to this disease in dogs.

Blood disorders: Anemia, thrombocytopenia, and neutropenia have been described as a result of phenobarbital administration (Jacobs et al., 1998). It is rare, and probably idiosyncratic, but should be considered in a patient that shows signs of a blood disorder in association with phenobarbital treatment.

Effect on hormones: Phenobarbital administration may alter thyroid hormone levels (Gieger et al., 2000; Kantrowitz et al., 1999). After multiple doses, it may decrease thyroxine (T_4) and free T_4 in dogs, but not T_3 (Kantrowitz et al., 1999). In one study, T_3 was increased slightly (Müller et al., 2000b). Thyroid stimulating hormone (TSH) has been mildly elevated in some studies, but not others (Müller et al., 2000b; Gieger et al., 2000). Thyroid hormones and TSH return to normal within 4 weeks after discontinuing phenobarbital treatment. The mechanism for the effect on thyroid hormone is probably via an increase in T_4 metabolism induced by phenobarbital (Kantrowitz et al., 1999; Gieger et al., 2000).

Corticosteroid metabolism: In experimental animals and humans phenobarbital accelerates steroid metabolism. This may decrease the therapeutic effects of corticosteroid administration. However, there is no evidence that phenobarbital treatment induces adrenal disease in dogs. The effect of phenobarbital administration to dogs on adrenal cortex diagnostic tests has been examined (Dyer et al., 1994; Chauvet et al., 1995). Phenobarbital administration to dogs did not affect response to exogenous adrenocorticotropic hormone (ACTH) or the levels of endogenous ACTH.

Clinical Use

Phenobarbital is considered the first drug of choice for long-term treatment of seizure disorders in dogs and cats (Podell et al., 2016). Its efficacy has been estimated to be approximately 60–90%. It also has been used to treat some behavior disorders in dogs and cats. Daily dosages of 4–16 mg/kg/day have been used, but dogs are usually started on 2.5–3 mg/kg q 12 h and adjusted up to 6–8 mg/kg q 12 h, gradually – if necessary. Even though the half-life is long in dogs, most animals are initially treated with phenobarbital on an every-12-hour schedule to decrease side effects and ensure minimal peak–trough fluctuations. In some patients, however, once-a-day dosing may be effective.

The typical starting dose for cats is 1.5–2.5 mg/kg, q 12 h, PO (Quesnel et al., 1997; Platt, 2001; Finnerty et al., 2014). In some cats, doses are increased to 2–4 mg/kg, q 12 h, PO. A common dose in cats is 7.5 mg per cat, q 12 h (half of a 15-mg tablet), and the dose may be adjusted by 7.5-mg increments.

In horses, the initial doses are 11 mg/kg q 24 h. When chronic therapy has been needed the dose may be increased to 25 mg/kg q 24 hr.

Treatment of status epilepticus: Phenobarbital may be injected in animals for the treatment of status epilepticus. One of the consensus documents (Bhatti et al., 2015) recommends a loading dose of 15–20 mg/kg IV, or IM, followed by additional doses in increments of 3–5 mg/kg over 24–48 hours until seizure control is maintained, or therapeutic plasma/serum concentrations are attained. One author recommends that in naïve animals (not previously on anticonvulsant maintenance medication), a loading dose of 12 mg/kg IV should be used, followed by 2–4 mg/kg increments every 20–30 minutes until seizure control is achieved (Muñana, 2013). Phenobarbital infusions also have been administered at the rate of 3–6 mg/dog per hour, IV (Bateman and Parent, 2000). Phenobarbital does not cross the blood–brain barrier as fast as diazepam; therefore, it may take several minutes for an anticonvulsant effect to treat status epilepticus. In severe cases, pentobarbital has been administered to effect, starting with doses of 4–20 mg/kg IV. Pentobarbital is not a good anticonvulsant; its use in this instance is simply to anesthetize the patient until additional therapy can be provided.

Formulations: Typically, phenobarbital tablets are the preferred dose formulation. There is also an injection formulation for IV use, and a liquid elixir. Because the elixir is bitter for many animals, phenobarbital occasionally has been compounded into an oral liquid for administration to small dogs and cats. These compounded formulations have been stable for 115 days.

Because cats may be difficult to medicate orally, a compounded transdermal formulation has been prepared in a polymer gel or proprietary compounding base to enhance absorption across the skin and applied to the ears of cats (Gasper et al., 2015; Krull et al., 2014). However, in one study the formulation in a pluronic lecithin organogel (PLO) matrix had inconsistent and low potency and at a dose of 6 mg/kg twice daily produced measurable, but subtherapeutic, plasma concentrations in cats (Krull et al., 2014). Another study using a compounded transdermal formulation showed that effective concentrations can be achieved in cats if a high dose of 9 mg/kg every 12 hours was administered (Gasper et al., 2015).There are no commercial forms available for transdermal application. Consistency of the formulations and long-term safety have not been evaluated.

Clinical Monitoring

To adjust dose, monitor compliance, and assess toxicity it is the usual practice to monitor plasma or serum phenobarbital concentrations regularly during therapy. Assays are available at most diagnostic laboratories. This can be done initially after the first 2 weeks of starting treatment and then every 6–12 months, or as needed to assess treatment. The recommended plasma/serum therapeutic concentration for dogs is 15–40 μg/ml (65–180 mmol/l). Some neurologists list this range as 20–45 μg/ml and 15–45 μg/ml, and a consensus panel listed 25–35 μg/ml (Bhatti et al., 2015) and 15–35 μg/ml (Podell et al., 2016) as the effective range. If dogs are also receiving bromide (see Section Bromide (Potassium and Sodium Bromide)), phenobarbital concentrations in the range of 10–36 μg/ml have been reported as therapeutic (Trepanier et al., 1998).

For cats, the plasma concentrations can be variable, but the optimum range has been cited as 23–30 μg/ml (Quesnel et al., 1997). In another study in cats, seizure control was achieved in 93% of cats when concentrations were between 15 and 45 μg/ml (Finnerty et al., 2014). In the cited study by Quesnel et al. (1997), cats were more unstable than dogs, possibly because of more serious CNS disease and required more frequent monitoring.

Because phenobarbital has such a long half-life, fluctuations between plasma peaks and troughs are minimized when it is administered on an every-12-hour schedule. Therefore, the timing of samples usually makes no difference when a sample is collected during a 12-hour schedule for the assessment of plasma phenobarbital concentrations (Levitski and Trepanier, 2000). However, in one study the trough concentrations were lower than at other times during the day when dogs received greater than 10 mg/kg per day, and they recommended always monitoring each individual patient at the same time after dosing to obtain consistent results (Monteiro et al., 2009).

Primidone

Primidone (Mylepsin, Mysoline) is a barbiturate derivative metabolized to phenobarbital and phenylethylmalonamide (PEMA). Primidone and its two major metabolites have anticonvulsant activity but at least 85% of the pharmacological activity is derived from phenobarbital. Primidone has been around for a long time and was once more widely used in dogs (but rarely in cats). But, since the 1980s, there has been little new information generated for primidone and its use has declined.

Pharmacokinetics

In addition to the differences in potency, PEMA and primidone do not persist in the plasma for as long as phenobarbital, which raises questions regarding the activity of PEMA and primidone throughout a long dosing interval (Frey et al., 1979). When primidone was administered to dogs, the half-life (mean value) of PEMA was 7.1 hours, the half-life of primidone was 1.85 hours, and the half-life of phenobarbital was 41 hours (Yeary, 1980). After multiple dosing in this study the primidone concentrations decreased, probably caused by accelerated metabolism.

Clinical Efficacy

Although seizures can be controlled with primidone in dogs (Schwartz-Porsche et al., 1982), primidone has little advantage over phenobarbital. Control of seizures in dogs is correlated with the plasma concentrations of phenobarbital, rather than primidone (Cunningham et al., 1983). In a comparison between primidone and phenobarbital in epileptic dogs (Schwartz-Porsche et al., 1982), there was no significant difference between phenobarbital and primidone with respect to seizure control, and primidone appeared more likely to induce liver injury than phenobarbital. The authors concluded that phenobarbital, rather than primidone, should be the drug of first choice for treatment of canine epilepsy.

However, there may be rare cases that respond to primidone when phenobarbital alone has not been effective (1 out of 15 according to Farnbach, 1984). Primidone is more expensive than phenobarbital, but it is not classified as a controlled drug in the USA; therefore, it is not subject to the same degree of regulation as phenobarbital.

Adverse Effects

Most adverse effects and side effects are the same as those listed for phenobarbital; however, primidone administration may be associated with a higher incidence of hepatotoxicity. Hepatic necrosis, fibrosis, and cirrhosis have been associated with chronic use of primidone (Bunch, 1989). Intrahepatic cholestasis has occurred in dogs in which primidone was combined with phenytoin.

Manufacturers do not recommend its use in cats; in fact, it is stated on the package insert that its use should be cautioned in cats. However, studies in cats have demonstrated that primidone is probably safe.

Clinical Use

Initial dosages in dogs are 3–5 mg/kg q 8–12 h, but have been increased up to 12 mg/kg q 8 h. If one is converting a patient from primidone to phenobarbital or vice versa, the conversion is the following: 65 mg phenobarbital = 250 mg primidone.

Therapeutic Monitoring

The effects of primidone are associated with the concentrations of phenobarbital; therefore, clinical monitoring should rely on phenobarbital concentrations (see recommended concentrations listed in Section Phenobarbital, Clinical Monitoring). Measurements of PEMA or primidone are not available in most laboratories.

Phenytoin

Phenytoin (Dilantin, formerly diphenylhydantoin) is one of the most commonly prescribed anticonvulsants in human medicine. In veterinary medicine, its use is rare because of pharmacokinetic differences and susceptibility to adverse effects. It is *not* a recommended anticonvulsant for dogs or cats. It is used occasionally in horses for muscle disorders or as an antiarrhythmic agent.

Mechanism of Action

Phenytoin stabilizes neuronal membranes and limits the spread of neuronal or seizure activity from the focus. It blocks inward movement of Na^+ and stabilizes excitable tissue (this drug is also used as a Class I antiarrhythmic agent, see Chapter 22). Phenytoin also decreases Ca^{+2} inward flow during depolarization, thus, inhibiting Ca^{+2}-dependent release of neurotransmitters (presynaptic).

Pharmacokinetics

There are important interspecies differences among animals and between animals and humans that cause difficulty in maintaining effective concentrations. Phenytoin is eliminated much more rapidly in dogs than in people (Frey and Löscher, 1985). Because of its rapid elimination, phenytoin is not recommended as an anticonvulsant in small animals. In cats, the elimination is very slow, and toxicity has been a concern. In horses the pharmacokinetics have been described (Soma et al., 2001), but are highly variable among horses. The half-life was approximately 12–13 hours, depending on the route of administration, but the oral absorption ranged from 14 to 85% among horses.

Clinical Use

Phenytoin is available in 25 mg/ml oral suspension; 30 and 100-mg capsules (sodium salt); and 50 mg/ml injection (sodium salt). For dogs, these formulation sizes result in an impractical dose (several capsules per day) for a large-size dog. Nevertheless, a dose recommended for dogs is 20–35 mg/kg q 8 h and the antiarrhythmic dose is 30 mg/kg q 8 h PO or 10 mg/kg IV over 5 minutes. It should not be used in cats.

Horses: Phenytoin has been used in horses for tying-up syndrome. This is a use primarily employed on the racetrack in athletic horses. The use is not related to the anticonvulsant property. In horses, at high doses recumbency and excitement have been observed. Sedation in horses may be an initial sign of high plasma phenytoin concentrations. Phenytoin pharmacokinetics are highly variable in horses, particularly the extent of absorption (Soma et al., 2001). Because of this variability it is difficult to maintain consistent plasma concentrations. Monitoring plasma concentrations may be necessary to adjust the dose to maintain an optimum level and prevent adverse effects. Suggested doses for horses are to administer an initial bolus of 20 mg/kg q 12 h PO, for four doses, followed by 10–15 mg/kg q 12 h PO. A single IV dose in horses of 7.5–8.8 mg/kg can be used, followed by oral maintenance doses.

Therapeutic Monitoring

Therapeutic drug monitoring can be performed; however, therapeutic concentrations have not been established for dogs and cats. Effective plasma concentrations listed for people are between 5 and 20 μg/ml. In the absence of other data, an average peak concentration of 15 μg/ml has been used as a target for animals. In horses, effective plasma concentrations are 5–20 μg/ml (average 8.8 μg/ml ± 2 μg/ml) (Soma et al., 2001). Therapy should be aimed at producing concentrations above 5 μg/ml in horses, or a peak concentration of 15 μg/ml.

Valproic Acid (Valproate)

Valproic acid (Depakene®) has been used in dogs primarily when they are refractory to other medications. The use has declined after other drugs became available (see Sections Zonisamide, Gabapentin, and Levetiracetam), but it has been used in some dogs successfully. The mechanism of action is unknown. It may interfere with GABA metabolism.

Pharmacokinetics

Absorption from oral valproate is high in dogs, with values for the immediate-release tablets of 100% (Bialer et al., 1984) and sustained-release preparations of approximately 80%. The half-life in dogs is 1.0–2.8 hours (15–20 hours in people), or approximately 1.4 hours in another study in dogs (Bialer et al., 1984). This short half-life severely limits its therapeutic effectiveness unless frequent dosing schedules are used or sustained-release products are employed. But, some evidence suggests that the anticonvulsant effects of valproate persist long after the drug is eliminated from the plasma.

Clinical Use

According to some studies in dogs (Bialer et al., 1984; Bialer, 1992), the sustained-release tablets provide a more sustained plasma concentration than conventional tablets. However, half-life and extent of absorption may be different between valproate pharmacokinetics in dogs and humans; therefore, the human extended-release dosage forms may be applied to dogs, but should be administered more frequently than in humans (Bialer, 1992; Bialer et al., 1986).

Divalproex sodium is composed of equal parts of valproic acid and valproate. It is pharmacologically equivalent to valproic acid and is available in delayed-release tablets of 125 and 500 mg (Depakote®, Epival®).

Although valproate is not commonly used, some neurologists have reported it to be "reasonably effective" and well tolerated. Combining this drug with another anticonvulsant such as phenobarbital may increase its efficacy. Dosages that have been used in dogs range from 75 to 200 mg/kg q 8 h when used alone, or 30 to 40 mg/kg/day with phenobarbital (phenobarbital dose is usually decreased). Drug assays exist for therapeutic monitoring, but this is not commonly performed for animals. The therapeutic concentrations are reported to be 350–830 μmol/ml (50–120 μg/ml), but the true "therapeutic range" for valproate in animals is not known.

Imepitoin

Imepitoin is approved for treating epilepsy in dogs in Europe, but not the USA. In Europe, the brand name is Pexion, it was approved in 2013, and is considered a first-line treatment for idiopathic epilepsy in dogs. Veterinarians in the USA have not been familiar with imepitoin.

The mechanism of action of imepitoin is to decrease seizures by potentiating the $GABA_A$ receptor-mediated inhibitory effects. Although structurally it is not classified as a benzodiazepine, these actions are similar to diazepam and other benzodiazepines (Figure 17.1). The similar action to benzodiazepines is caused by its low-affinity binding for the benzodiazepine binding site of $GABA_{A,}$ but it has 600 times less affinity for the binding site compared to diazepam. It also may have a weak calcium channel blocking effect, which may contribute to clinical effects.

Clinical Use in Dogs

The approved dose for dogs of imepitoin is 10–30 mg/kg oral twice daily. It is available in 100 and 400-mg tablets, which can be split if necessary. It is recommended to start with 10 mg/kg, then increase dose to obtain the desired response. There have been no guidelines thus far for plasma/serum concentration monitoring; therefore, dose adjustment should be made on the basis of clinical response. The dose can be increased by 50 to 100% increments up to a maximum dosage of 30 mg/kg administered twice daily.

In a US field trial it was compared to primidone in dogs. Imepitoin efficacy was lower than primidone, but was judged to be not inferior, and produced fewer adverse effects in dogs compared to primidone. In the field trials that led to the approval for use in Europe, the efficacy of imepitoin was compared to phenobarbital in dogs with idiopathic epilepsy. The European agency concluded that although there was lower overall efficacy for imepitoin compared to phenobarbital, some dogs were well-controlled with imepitoin treatment. They stated that although efficacy may not be complete with imepitoin treatment in dogs, it is considered a “suitable treatment” in some dogs because of its safety profile. The ACVIM consensus panel (Podell et al., 2016) gave it a high rating for use as single-drug monotherapy, but a rating of low as an add-on medication. In the published study in which it was compared with phenobarbital for idiopathic epilepsy in dogs (Tipold et al., 2015), there were significantly fewer adverse events with imepitoin compared to phenobarbital. Although the efficacy was lower for imepitoin than phenobarbital, the analysis showed imepitoin to be statistically noninferior. It has not been evaluated for treatment of status epilepticus. The use as an add-on treatment with other drugs has not been sufficiently tested, except in a pilot study in which there were add-on benefits (Rieck et al., 2006). There have been no studies reported in cats or other animals.

Pharmacokinetics

Studies in dogs indicate that half-life of imepitoin in Beagle dogs was relatively short compared to other anticonvulsant drugs. The half-life was 1.5–2 hours, but it can be administered only twice daily (Rundfeldt and Löscher, 2014). Most of the elimination is by the feces. The authors of the study suggested that it may have shorter half-life in Beagle dogs than other breeds, but those studies may not be representative of clinical patients. Concentration profiles were not much different in fed versus fasted Beagle dogs. The total area-under-the-curve (AUC) was higher in the fasted dogs, even though this may not be enough of a difference to be clinically relevant. The protein binding was 56–57%, but no protein-binding drug interactions have been identified.

Adverse Effects

The most common adverse effects from imepitoin reported in field trials were lethargy/sedation, polyuria/polydipsia, and increased appetite. Most reactions are mild and generally transient. Other reported effects are hyperactivity, hypersalivation, emesis, ataxia, diarrhea, and sensitivity to sound. In the clinical studies it produced fewer adverse effects compared to primidone or phenobarbital. In clinical studies, although phenobarbital was associated with increasing levels of the liver enzymes ALT, ALP, aspartate aminotransferase (AST), γ-glutamyl transferase (GGT), and glutamate dehydrogenase (GLDH), none of these enzymes increased with imepitoin. Although it is a partial agonist for the $\mathrm{GABA_A}$ binding site, there is no evidence of tolerance or dependence that develop from repeated administration of imepitoin.

Drug Interactions

Imepitoin is metabolized by the liver enzymes; therefore, interactions with other drugs that affect cytochrome P450 enzymes are possible, but have not been studied in dogs. It did not affect activity of most human P450 isoforms but inhibition of CYP1A1 is possible (Rundfeldt and Löscher, 2014). Imepitoin had no effect on enzyme induction compared to phenobarbital (Rundfeldt and Löscher, 2014). It is a partial agonist for the benzodiazepine receptor, but there is no evidence that it will prevent the activity of coadministered benzodiazepines used for acute treatment (e.g., status epilepticus). In a pilot study (Rieck et al., 2006), there was no evidence of interaction with coadministered phenobarbital in dogs.

Imepitoin is poorly soluble and dissolves more readily at an acid pH. Therefore it is possible that suppressing acid in the stomach (for example with proton pump inhibitors such as omeprazole) could interfere with oral absorption.

Benzodiazepines

Diazepam

Diazepam belongs to the class of benzodiazepines (Figure 17.1). Diazepam (Valium) is an important drug for treating acute seizures and status epilepticus, but is not practical for long-term therapy. Diazepam along with other benzodiazepines is discussed in Chapters 9, 14, and 18.

Mechanism of Action

The mechanism of action as an anticonvulsant is caused by the action to hyperpolarize neurons and suppresses neuronal activity. Benzodiazepines accomplish this action through interaction with the inhibitory neurotransmitter GABA. The $\mathrm{GABA_A}$ receptor subunit is the most prominent. It is a ligand-gated chloride ion ($\mathrm{Cl^-}$) channel and the site of action of many neuroactive drugs, such as benzodiazepines, barbiturates (discussed previously), and some anesthetics. Benzodiazepines act on $\mathrm{GABA_A}$ by binding to a specific site that is distinct from that of GABA binding. They do not activate GABA receptors directly (which is the action of barbiturates) but modulate the effects of GABA. From the interaction with $\mathrm{GABA_A}$, the frequency of GABA-activated $\mathrm{Cl^-}$ channels is increased.

Pharmacokinetics

In humans the half-life is 43 hours (mean) but may range from 24 to 48 hours, and as high as 60 hours. It has a low hepatic extraction rate compared to dogs. In dogs, the half-life is much shorter (Frey and Löscher, 1985), with a high hepatic extraction rate, which makes this drug unsuitable for most dogs for chronic anticonvulsant therapy. The half-life from IV administration has been reported to be as rapid as 15 minutes and as long as 3.2 hours, depending on the study (Papich and Alcorn, 1995). Diazepam is rapidly converted to two metabolites. It is first demethylated to *N*-desmethyldiazepam (also called nordiazepam), then to oxazepam. The metabolites are eliminated more slowly than diazepam and have equal to one-third the potency of diazepam, depending on the study used to determine potency. The half-life of the desmethyldiazepam metabolite is 2.2–3.6 hours and of oxazepam is 3.83–5.7 hours. In cats, the half-life of diazepam is 5.5 hours, with the half-life of the metabolite 21 hours. Diazepam is highly lipophilic, more so than other drugs in this class. Its high lipophilicity produces high concentrations in the CNS rapidly after IV injection, which is why this drug is so useful in emergency situations to treat status epilepticus.

Clinical Use

Diazepam is usually the first drug of choice for treating status epilepticus because it is distributed rapidly to the CNS after IV administration. But, it is unsuitable for chronic treatment in dogs because frequent administration is required. There are also other disadvantages: tolerance develops with repeated administration, animals may develop dependence, and there is abuse potential (by animal owners).

For emergency use in dogs, diazepam is administered IV at a dose of 5–20 mg/animal (diazepam concentration is 5 mg/ml). The equivalent dose is approximately 0.5 mg/kg. Diazepam is lipophilic (Log P 2.76) and crosses the blood–brain barrier quickly for a rapid effect. It has a short duration of action, but the metabolites (desmethyldiazepam and oxazepam) are active and have longer half-lives. Repeated doses may be administered to patients that continue to seize, but some veterinarians have utilized constant rate infusions. Infusion doses are in the range of 0.5 mg/kg per hour, IV (Bateman and Parent, 2000). One must be aware of the potential for diazepam to adsorb and absorb to plastic IV infusion sets when administered as an IV infusion.

Rectal administration: Rectal administration is a convenient alternative to IV dosing in an animal with status epilepticus when intravenous or oral administration is not practical. Diazepam is rapidly absorbed by this route (Papich and Alcorn, 1995) and has been effective in dogs for at-home treatment of cluster seizures (Podell, 1995). The 5 mg/ml injectable solution has been instilled in the rectum with a syringe starting at a dose of 0.5–1 mg/kg, and as high as 2 mg/kg. The study by Wagner et al., (1998) concluded that 2 mg/kg via the rectal route was appropriate, even if dogs had been receiving chronic phenobarbital. A small plastic teat cannula attached to the end of the syringe has been used to facilitate administration. A formulation in gel has been made available for this use (Dreifuss et al., 1998), but it is more expensive.

Other routes of administration: Other routes have been used, such as intranasal and mucosal (gingival). The only one of these routes that has been examined well in dogs was the nasal route. Intranasal administration of 0.5 mg/kg to Greyhounds produced plasma concentrations of diazepam and metabolites in an effective range for seizure control (Platt et al., 2000). A more detailed study that analyzed specific metabolites showed that after intranasal administration of 0.5 mg/kg to dogs, effective concentrations were reached within 5 minutes (Musulin et al., 2011). Although diazepam had a short half-life of approximately 2 hours in that study, the half-life of the active metabolites (desmethyldiazepam and oxazepam) was much longer. Both these studies conclude that intranasal administration may be an effective method of administration to dogs with status epilepticus.

Diazepam in cats: In cats diazepam has a longer half-life than dogs and has been used as a drug to control seizures, treat behavioral problems, and stimulate appetite (see Chapter 18). In cats, dosages of 0.25–0.5 mg/kg q 8 h, PO are used. This dose may be increased to 1–2 mg/kg q 8 h for seizure control. Adverse hepatic effects (discussed in Section Adverse Reactions and Tolerance) have caused a decline in the long-term administration to cats.

Adverse Reactions and Tolerance

Some neurologists have observed that patients may become refractory to chronic treatment (tachyphylaxis) because there may be a feedback decrease in benzodiazepine receptor synthesis or a decreased synthesis of GABA. Tolerance in dogs may be seen after 1 week of dosing. After long-term administration, withdrawal should be done gradually and carefully because some dependence occurs. Signs of withdrawal may include increased anxiety and tremors.

Hepatic toxicosis in cats: Hepatic necrosis in cats has been reported from oral administration of diazepam (Center et al., 1996). This reaction can be severe and fatal. The liver injury usually appears after the initial 5 days of treatment. Diazepam-induced hepatic injury in cats may be caused by slow metabolism to the metabolite temazepam, and inhibition of the bile salt export pump, causing accumulation of bile acids in hepatocytes (Van Beusekom et al., 2015). Because of this reaction, other benzodiazepines should be considered in cats.

Other Uses

Skeletal muscle relaxant: Benzodiazepines may act as muscle relaxants by inhibiting certain spinal pathways – most likely through GABA effects – or by directly depressing motor nerve and muscle function. Examples of their use have been relaxation of urethral skeletal muscle in cats following obstruction and relaxation of skeletal muscle associated with spinal disk disease. Their clinical efficacy as muscle relaxants has been questioned.

Treatment of anxiety, aggression, and behavioral disorders in small animals: Because benzodiazepines are common drugs in human medicine for emotional disorders – anxiety, stress, phobias, aggression, insomnia, etc. – they have also been administered to dogs and cats for a large variety of disorders with varied success. Other benzodiazepines, such as alprazolam, are usually used for treatment of anxiety disorders in animals. A discussion of the use of alprazolam and other benzodiazepines for behavior problems can be found in a review paper (Simpson and Papich, 2003) and in Chapter 18.

Diazepam-induced eating in anorexic cats: Benzodiazepines stimulate the appetite in anorexic cats (less effective in dogs). Diazepam has been administered for this purpose at dosages of 0.04–0.05 mg/kg, IV. Oral oxazepam also has been used for this indication.

Midazolam

Midazolam does not have a role in chronic management of seizure disorders in animals, but has been used to treat status epilepticus. It is also frequently used as an anesthetic adjunct, which is discussed in Chapter 14. Midazolam acts in the same manner as other benzodiazepines. An important difference between midazolam and diazepam is the solubility. At a pH <4 it exists in an open-ring form allowing preparation of water-soluble salts. It is capable of achieving an equilibrium in this soluble form because the solution (midazolam hydrochloride) has pH of approximately 2.5 to 3.7, adjusted with hydrochloric acid and, if necessary, sodium hydroxide. The aqueous midazolam preparation produces little tissue irritation and can be administered intramuscular or intravenous in aqueous solutions. Following injection, the more neutral pH of the body (pH above 5.5) converts midazolam to a closed-ring form, which facilitates its lipid solubility. It is almost as lipophilic as diazepam at this pH (Log P 2.68) and readily crosses the blood–brain barrier for treatment of status epilepticus.

The pharmacokinetics of midazolam have been studied (Schwartz et al., 2013) with pharmacokinetics of other studies summarized in that report. Because of favorable absorption and ease of administration, midazolam has become a useful emergency treatment for seizures at a dose of 0.2 mg/kg (solution is 5 mg/ml). The systemic availability from an intramuscular (IM) injection in dogs was 50% (Schwartz et al., 2013). However IM absorption was variable among dogs and a dose higher than 0.2 mg/kg should be used to ensure high enough concentrations for treating seizures. Unlike diazepam, absorption following rectal administration of midazolam was low and unlikely to be effective (Schwartz et al., 2013). Intranasal midazolam was absorbed in dogs well enough to produce 70% efficacy within 47 seconds for treating status epilepticus (Charalambous et al., 2017). The half-life in dogs is short (approximately 1 hour) and additional doses may be needed if prolonged seizure control in the hospital is needed.

Clonazepam

Clonazepam (Klonopin) is another benzodiazepine with a mechanism of action similar to diazepam, but it is more potent. There is anecdotal evidence that it is a good anticonvulsant in dogs that are refractory to phenobarbital therapy, but this should be considered only as a last resort. Clonazepam undergoes saturable (zero order) elimination. As the dose is increased, or if animals are dosed for longer than 1 week, the half-life increases (e.g., from 1.5 hours to 3 hours). As for other antiepileptic drugs, the half-life is much longer in humans compared to dogs. The starting dose recommended for dogs is 0.5 mg/kg, two to three times a day (Frey and Löscher, 1985). In cats, clonazepam has been used as an alternative to diazepam to avoid the risk of hepatotoxicity. The starting dose is 0.5 mg/kg once or twice daily in cats.

Withdrawal syndrome is possible from chronic therapy with clonazepam. If dogs are receiving clonazepam and there is abrupt cessation of therapy, signs of acute withdrawal can be seen, which include listlessness, weight loss, pyrexia, and recumbency. Cessation of this drug should be tapered over a period of 1 month to minimize these effects.

Clorazepate

Clorazepate (Traxene) is another benzodiazepine that has also been considered for treating seizures in animals. But because of lack of published data on efficacy and availability of other choices, clorazepate should be considered only after other treatments have been attempted. Although there is no published information regarding its efficacy in dogs, anecdotal reports indicate that it may be effective in some refractory cases.

Clorazepate is hydrolyzed to desmethyldiazepam (nordiazepam), an active metabolite of diazepam. The half-life of desmethyldiazepam is 3–6 hours in dogs (30–100 hours in man) (Frey and Löscher, 1985; Papich and Alcorn, 1995; Musulin et al., 2011). Dosages of 2 mg/kg every 12 hours may provide therapeutic concentrations in dogs. Therapeutic plasma concentrations of desmethyldiazepam are usually greater than 1 μg/ml.

As mentioned for clonazepam, clorazepate is also used in cats as an alternative to diazepam. There has not been much experience in cats, but doses in the range of 3.75 to 7.5 mg per cat once daily have been used.

Felbamate

Felbamate (Felbatol) has been used in dogs that are refractory to other anticonvulsant drugs. Since there is less experience with felbamate and other drugs are now available, it is usually considered as a last resort (Bhatti et al., 2015). The action is not completely understood, but it is an antagonist at the *N*-methyl-D-aspartate (NMDA) receptor–ionophore complex. Antagonism of NMDA may block effects of excitatory amino acids and suppress seizure activity (increase seizure threshold and decrease seizure spread). It addition there may be some neuroprotective effects from antagonizing excitatory amino acids. This unique mechanism of action has been used to argue that it may be considered in patients that are refractory to other anticonvulsant drugs.

Pharmacokinetics

In dogs the half-life has been reported to be approximately 5–6 hours (Adusumalli et al., 1992). The pharmacokinetics have not been examined in other animals.

Clinical Use

The initial dose in dogs is approximately 15–20 mg/kg q 8 h, orally. In practice, the starting dose is 200 mg/dog orally q 8 h (small dogs) and increases by 200 mg per week until seizures are controlled to a maximum of 600 mg/dog q 8 h. For large dogs, start with 400 mg/dog q 8 h and increase gradually to a maximum of 1,200 mg/dog q 8 h. (This dose range is approximately 15–65 mg/kg q 8 h.) Felbamate has induced its own metabolism in dogs; therefore, there may be a need to increase doses as therapy progresses. Felbamate human formulations (Felbatol®) are available in 120/ml oral liquid and 400 and 600 mg tablets.

Adverse Effects

Adverse reactions have not yet been documented with clinical use in dogs, but it has not been used frequently. It does not seem to produce the sedation and behavior changes that other drugs have been known to cause. Toxic signs have not been seen in dogs unless the dose exceeded 300 mg/kg/day. In people there are strong warnings on the label. Use in humans has caused aplastic anemia and severe hepatic failure resulting in death. In people it has been shown to increase phenobarbital concentrations by 20–30% so careful monitoring of phenobarbital concentrations is suggested.

Gabapentin

Gabapentin (Neurontin and generic brands) has been used in dogs and cats to control seizures when other anticonvulsant drugs have not been effective, or when others have been too toxic. It is ordinarily used as an add-on treatment; there are not studies reported in which it has been used as monotherapy. Despite this use, there is little evidence available in dogs or cats to show that it is effective. Subsequently, it was not included as a recommended drug by the ACVIM consensus group (Podell et al., 2016).

Gabapentin is a structural analog of GABA, but the mechanism of action as an anticonvulsant is not entirely known. It does not interact with GABA receptors and is not a GABA agonist, and does not affect GABA uptake or degradation. It also does not interfere with sodium-dependent channels or exhibit affinity for other neurotransmitter receptors such as those affected by benzodiazepines, glutamate, dopamine, or NMDA.

The same action that is responsible for its use for treating neuropathic pain may also explain its anticonvulsant activity. The mechanism of action for treating neuropathic pain appears to be via blocking calcium-dependent channels. Gabapentin inhibits the alpha-2-delta (α_2 Δ) subunit of the N-type voltage-dependent calcium channel on neurons. After binding to the α_2 Δ subunit it reduces calcium influx that is needed for release of neurotransmitters – specifically excitatory amino acids – from presynaptic neurons. This channel becomes up-regulated when nerves are stimulated, such as in epileptic conditions or associated with neuropathology. Blocking the channels has little effect on normal neurons, but appears to suppress stimulated neurons.

Pharmacokinetics

Gabapentin is absorbed in dogs with systemic oral availability that is practically complete (80% or greater) and is not affected by food. Compared to the elimination in people, gabapentin has a short half-life of 2–4 hours in dogs (Radulovic et al., 1995; KuKanich and Cohen, 2011). The systemic clearance resembles renal clearance values in dogs of 2–3 ml/kg/min (Radulovic et al., 1995), which has led to the conclusion that gabapentin is excreted by renal mechanisms and does not rely on hepatic biotransformation, except for small biotransformation in dogs to

N-methylgabapentin. Therefore, it has been used in animals when hepatic disease or impaired metabolism is a concern. Protein binding in dogs is negligible (Radulovic et al., 1995).

The pharmacokinetics in cats was studied at a dose of 4 mg/kg IV and 10 mg/kg oral (Siao et al., 2010). The oral absorption was 89%, but was variable among cats. The average oral half-life was approximately 3 hours (similar to dogs). This study predicted an effective oral dose in cats of 3 mg/kg every 6 hours.

Because of interest in using gabapentin in horses for chronic pain, pharmacokinetics were studied after an oral administration of 5 mg/kg (Dirikolu et al., 2008). Oral clearance was high and the half-life was 3.4 hours (similar to dogs and cats). In another study the oral absorption of 20 mg/kg was only 16%, but the half-life was longer (approximately 8 hours), and the horses tolerated it well (Terry et al., 2010).

Lipophilicity and penetration: The volume of distribution is small compared to other anticonvulsants (0.158 l/kg) (Radulovic et al., 1995), an octanol/water partition coefficient (Log P) of only −1.10 and a partition coefficient at pH 6–8.5 (Log D) of only −1.44. These values indicate that lipophilicity is lower than what is needed to penetrate the blood–brain barrier. Therefore, gabapentin must rely on a carrier for penetration to the CNS. Gabapentin mimics an α-amino acid and utilizes the large amino acid transporter to penetrate the brain (Jolliet-Riant and Tillement, 1999).

Clinical Use

Gabapentin is available in 100, 300, and 400-mg capsules; 100, 300, 400, 600, and 800-mg scored tablets; and 50 mg/ml oral solution. The oral solutions contains xylitol, which is known to be toxic to dogs. The dose of oral solution administered to dogs must consider the amount of xylitol delivered, and avoid the use of this product if the dog is already receiving xylitol-containing medications.

Anticonvulsant use: Gabapentin is used primarily for refractory seizures that have not responded to other drugs. It has not been used alone as an anticonvulsant in animals. In people the dose varies from 900 to 1,800 mg per person per day, and up to 3,600 mg per day. In dogs the most common dose (derived empirically) has been 10–20 mg/kg orally, every 6–8 hours. A study in greyhound dogs predicted that a dose of 10–20 mg/kg oral, every 8 hours would produce concentrations at an effective level of 2 μg/ml (KuKanich and Cohen, 1011). In cats, it has been administered at a dose of 5–10 mg/kg per day orally, increasing the frequency to twice daily.

Use for treatment of pain: Another use of gabapentin in animals is for treatment of pain syndromes – particularly those identified with neuropathic pain. The mechanism responsible for this effect is via blockade of the calcium channels that may be up-regulated in painful neuropathic conditions, as discussed previously. This use stems from the experience in people in which gabapentin is used to treat neuropathic pain caused by postherpetic neuralgia (shingles), diabetic neuropathy, and other types of pain associated with neuralgia. Neuropathic pain is difficult to diagnose in animals, but may result from nerve pathology linked to another underlying disease. There are no well-controlled studies that have demonstrated effective neuropathic pain relief from gabapentin in dogs. A clinical postoperative pain study failed to demonstrate effectiveness (Wagner et al., 2010), and it failed to demonstrate a significant benefit in dogs for treating pain associated with intervertebral disc surgery (Aghighi et al., 2012). The use of gabapentin has also become popular in cats for treating chronic pain, but antinociceptive studies failed to demonstrate an effect (Pypendop et al., 2010). For neuropathic pain the dose used is 10–15 mg/kg q 8 h PO, and increased as necessary to levels similar to the anticonvulsant dose.

Gabapentin also was used to treat neuropathic pain in horses associated with laminitis and other conditions. After a dose of 2.5 mg/kg orally every 12 hours, gabapentin subjectively appeared to relieve pain in a horse that was secondary to surgery (Davis et al., 2007). It has also been used in racing horses, even though it is a Class 3 substance for race horses. Despite this growing interest for its use in horses, and few adverse effects reported, it had no significant effect for reducing lameness level compared to the placebo at an oral dose of 5 and 10 mg/kg three times daily in horses with chronic pain (Caldwell et al., 2015).

Adverse Effects

There have been no reported serious safety problems with gabapentin in animals during routine use. Sedation and ataxia are reported as the only significant adverse effects. In people a withdrawal syndrome from abrupt discontinuation has been described, but it is not reported in animals.

Pregabalin

Another drug related to gabapentin is pregabalin (Lyrica), but pregabalin is a classified as a controlled substance in the USA and gabapentin is not. It is used in people for neuropathic pain similar to gabapentin use. There is some information on its use in small animals, but it is limited. One study (Dewey et al., 2009) in a small number of dogs showed potential benefit as an adjunct drug in addition to phenobarbital and bromide for refractory epilepsy. The dose used in that study was 3–4 mg/kg oral every 8 hours.

The pharmacokinetics have been studied in dogs (Salazar et al., 2009) and were similar to people with a half-life of approximately 7 hours. Their study suggested a dose of 4 mg/kg every 12 hours would maintain the drug concentrations in an effective range (2.8–8.2 μg/ml) for treatment of pain and epilepsy. Preliminary studies on the pharmacokinetics in cats suggest a dose of 1–2 mg/kg every 12 hours and increased to 4 mg/kg every 12 hours orally (Cautela et al., 2010). Because oral absorption of gabapentin is low in horses (16%), pregabalin was studied to determine if absorption would be better. In horses after a dose of 4 mg/kg the oral absorption was 98% and half-life approximately 8 hours (Mullen et al., 2013). They predicted a dose of 4 mg/kg oral to horses every 8 hours would produce plasma drug concentrations in a range considered effective for people.

Levetiracetam

Levetiracetam (Keppra and generic) is another anticonvulsant drug used in people that has been adapted for use in dogs and cats. Levetiracetam is structurally and mechanistically different from other anticonvulsant drug classes (Figure 17.1). The mechanism of action of levetiracetam is not completely understood, but it has significant anticonvulsant activity in people for several types of seizures. It does not appear to affect membrane channels, GABA, membrane receptor activity, or glutamate receptor neurotransmission. Levetiracetam is capable of suppressing seizure activity without affecting normal neuronal excitability. One proposed mechanism of action is through binding to a presynaptic protein (synaptic vesicle 2a) and modulating voltage-gated calcium-dependent neurotransmitter release from vesicles in neurons. The site of binding is exclusively in the CNS, without any known peripheral effects.

The pharmacokinetics have been studied sufficiently to recommend dosing protocols to maintain plasma concentrations at a level that is considered effective in people (Isoherranen et al., 2001; Dewey et al., 2008; Patterson et al., 2008; Moore et al., 2010, 2011). It has good oral absorption, but a short half-life necessitating dosing at least every 8 hours. It has not been considered as monotherapy treatment for dogs or cats. It is usually used as an add-on, for patients with refractory epilepsy who are already receiving phenobarbital or bromide.

According to Isoherranen et al. (2001) levetiracetam is the ethyl analogue of piracetam, which has been used, among other things, for cognition enhancement in elderly people. Levetiracetam is the *S*-isomer of a chiral drug that exists in two enantiomers. Only the *S*-form, levetiracetam, has anticonvulsant activity. It has high solubility (>1 grams/ml), and octanol/water partition coefficient (Log D at 7.4) of −0.64.

Pharmacokinetics

In pharmacokinetic studies performed in dogs with IV, IM, and oral doses (Isoherranen et al., 2001; Dewey et al., 2008; Patterson et al., 2008; Moore et al., 2010, 2011), levetiracetam had 100% absorption from all routes of administration. Protein binding of levetiracetam is small (<10%); therefore, drugs or conditions that affect plasma protein will not alter levetiracetam pharmacokinetics. The volume of distribution ranged from 0.45 to 0.89 l/kg, depending on the study. With a half-life that ranged from 3 to 4.5 hours, it can be administered three times daily and maintain plasma concentrations that are within the range cited for humans as effective (Moore et al., 2010). After multiple doses for 6 days, there was no change in pharmacokinetic parameters (Moore et al., 2010).

Levetiracetam does not undergo significant hepatic metabolism, and it is mostly excreted unchanged in the urine. Although drug interactions with phenobarbital have been documented (see Section Adverse Effects and Interactions), liver disease is not expected to affect its pharmacokinetics. This drug has been one of the preferred drugs when it is anticipated that liver function may be altered by other drugs or diseases.

Pharmacokinetics in cats have demonstrated 100% oral absorption and a short half-life of approximately 3 hours (Carnes et al., 2011; Dewey et al., 2005); however, the half-life was variable among cats in a clinical study (Bailey et al., 2008).

Clinical Use

Levetiracetam is used clinically in dogs for refractory epilepsy when other drugs have not been effective, often as an add-on to phenobarbital and/or bromide therapy (Podell et al., 2016; Bhatti et al., 2015). Clinical studies have revealed some initial success, but long-term benefit is uncertain. In a small study (Volk et al., 2008), most dogs responded initially with a decrease in seizure frequency, but most responders had an increase in seizure frequency after 4–8 months of continued treatment. In a retrospective study (Packer et al., 2015), it was effective in most dogs administered either as maintenance treatment or as a pulse treatment, but in another clinical study, it was not effective in a small number of dogs compared to placebo when added to other anticonvulsant treatments in dogs with refractory epilepsy (Muñana et al., 2012). The ACVIM consensus panel gave it a low rating for monotherapy treatment but a better grade for add-on therapy.

Levetiracetam (Keppra and generic) is available in 250, 500, and 750-mg tablets. The usual human dose is 500 mg q 12 h. In dogs, the typical dose is 20 mg/kg orally, every 8 hours, which will maintain plasma concentrations within a range that is reported to produce anticonvulsant activity in people (Moore et al., 2010). In dogs receiving pretreatment with levetiracetam to prevent postoperative seizures from portosystemic shunt surgery, a dose of 20 mg/kg every 8 hours significantly reduced the risk of postoperative seizures and death (Fryer et al., 2011).

The effective plasma concentrations in dogs and cats are not known, but doses have been aimed at reaching the effective plasma concentrations in people of 12–46 μg/ml. There are also extended-release generic formulations for people that have been used in dogs. Although the prolongation of effective plasma concentrations was minor from the extended-release formulation, in some dogs it can be administered once every 12 hours and maintain plasma drug concentrations above the suggested therapeutic level. The extended-release tablets (500 and 750 mg) contains levetiracetam in a drug release controlling polymer. The tablets should not be crushed or broken so that extended-release properties are maintained. There is also an injectable formulation (100 mg/ml) that can be administered IV, and shown to be effective for emergency treatment of seizures (Hardy et al., 2012). The initial IV dose in the study was 30 mg/kg, and could be safely increased to 60 mg/kg. Rectal administration of the IV solution also has been explored for dogs. Although the rectal dose must be higher than oral administration (40 mg/kg for dogs), absorption from the rectal route can attain effective concentrations (Peters et al., 2014). In large dogs, this may be impractical because of the large volume required to administer an effective dose.

There has been limited evaluation of levetiracetam in a small number of cats (Bailey et al., 2008; Dewey et al., 2005). At 20 mg/kg q 8 h, orally, it was well tolerated and effective in some cats as an adjunct to phenobarbital treatment. To maintain effective concentrations, higher doses may be needed in some cats (Carnes et al., 2011). A syndrome known as audiogenic reflex seizures in cats has been described in which seizures may be triggered by high-pitch sounds (Lowrie et al., 2016). Apparently the cats with this form of seizures respond much better to administration of levetiracetam than phenobarbital. The efficacy for this syndrome may be related to levetiracetam's affinity for the synaptic vesicle that mediates the anticonvulsant effect.

Adverse Effects and Interactions

Few adverse effects have been reported in dogs and cats at the currently recommended dosages. Some dogs may show sedation, ataxia, restlessness, and gastrointestinal disturbances, but in these instances it was always administered with at least one other anticonvulsant. Intravenous levetiracetam has been administered to dogs at doses of 60 mg/kg without reported adverse effects (Dewey et al., 2008; Patterson et al., 2008). At high doses in excess of 600 mg/kg/day, nausea and vomiting in dogs has been observed, but no deaths. The most common adverse effect in cats was lethargy. In people, adverse effects are uncommon and may be idiosyncratic because there has been no relationship between plasma drug concentrations and adverse events (Contin et al., 2004).

Because it does not rely on extensive hepatic metabolism by cytochrome P450 enzymes, most drug interactions are uncommon. However, studies in dogs (Moore et al., 2011) showed a significant decrease in plasma concentration, shorter half-life and more rapid clearance of levetiracetam when it was administered with phenobarbital (enzyme induction by phenobarbital is discussed in Section Phenobarbital, Pharmacokinetics). Studies in people have shown that elderly people may have lower clearance, and some anticonvulsant drugs have induced a more rapid clearance (Hirsch et al., 2007; Contin et al., 2004; May et al., 2003). Lower clearance in the elderly was associated with decreased creatinine clearance.

Zonisamide

Zonisamide (Zonebran) is a human anticonvulsant that has been used in some canine epileptics and some cats that are refractory to other drugs. Zonisamide is chemically classified as a sulfonamide (Figure 17.1), but unrelated to other anticonvulsants. It has a high pKa of 10.2, a low molecular weight of 212.23, and solubility in water that is close to 1 mg/ml. It can suppress seizures induced by a variety of stimuli. Mechanism of action is unclear. It does not affect GABA-mediated mechanisms, but may block voltage-gated sodium and calcium channels (T-type calcium channels). The result of these actions is to stabilize neuronal membranes and suppress neuronal hyperactivity.

Pharmacokinetics

Because of growing interest for using zonisamide as an anticonvulsant in animals, more data have become available. In people, it has low (40%) protein binding, a high volume of distribution, low clearance (0.3 ml/kg/min), and half-life of 63 hours in plasma and 105 hours in red blood cells (RBCs). Levels are much higher in RBCs (eightfold higher), which are sometimes used for drug assays. It is excreted primarily in urine as the parent drug or as a glucuronide metabolite. It also undergoes *N*-acetylation. Because these two mechanisms – *N*-acetylation and glucuronidation – are deficient in dogs and cats, respectively, this may contribute to important pharmacokinetic differences in dogs and cats compared to people. Dogs are known to have adverse reactions to sulfonamide antimicrobial drugs, which may be related to their deficiency in *N*-acetylation metabolism (discussed in more detail in Chapter 32).

The disposition of zonisamide has been studied in dogs (Matsumoto et al., 1983; Boothe and Perkins, 2008). Oral absorption in dogs was 68%, with a half-life of 15–17 hours in plasma, depending on the study and route, but longer in red blood cells (44–46 hours in whole blood, and 91 hours in RBCs). Protein binding averaged 39.5% across a range of concentrations (Boothe and Perkins, 2008). In cats the mean half-life was longer than in dogs (31.5 hours) after a dose of 10 mg/kg (Hasegawa et al., 2008).

Clinical Use

In dogs, initial recommended doses of zonisamide in the range of 6–10 mg/kg twice daily orally have been recommended. But based on a pharmacokinetic study (Boothe and Perkins, 2008), these doses may be too high and they recommended a dose of 3 mg/kg oral, twice daily to reach concentrations in the desired range of 15 μg/ml. Phenobarbital may increase clearance (by approximately 50%). Therefore, if administered in combination with phenobarbital a higher dose of 7–10 mg/kg twice daily may be considered (Orito et al., 2008). It is available in 100-mg capsules.

It rarely used alone and is reserved for treating epilepsy in animals as an add-on to other drugs. In clinical studies with small numbers of dogs treated for refractory epilepsy along with other drugs, it has been effective in most dogs. The ACVIM consensus group rated it low for monotherapy treatment and moderate for add-on therapy. There have been three studies of efficacy reported in animals. In the study by Dewey et al. (2004) 12 dogs with refractory epilepsy were treated with an average dose of 8.9 mg/kg every 12 hours orally; 58% of dogs responded favorably, but 5 of 12 dogs actually had an increase in seizure frequency. In the study by von Klopmann et al. (2007) data from 11 dogs were evaluated. The dogs were administered 10 mg/kg twice daily for treatment of refractory epilepsy. Of 11 dogs, 9 were responders. In three of the dogs there was a decrease in response over time, which may reflect tolerance that develops with long-term treatment.

Zonisamide is typically not monitored clinically, but effective concentrations in dogs were reported to be above 12.6 μg/ml (Masuda et al., 1979). Neurotoxic effects in dogs were seen at concentrations above 96 μg/ml. In the study by Dewey et al. (2004) cited above, the effective concentrations were 10–40 μg/ml and similarly in the report by von Klopmann et al. (2007) were 15–38 μg/ml. Most veterinary neurologists now recommend a desired plasma concentration of 10–40 μg/ml based on extrapolation from people (Muñana, 2013; Bhatti et al., 2015; Podell et al., 2016). When measuring plasma drug concentrations, one should be sure to avoid hemolysis of red blood cells in the sample.

In cats there has been more limited use of zonisamide compared to dogs. A dose of 5 mg/kg oral, every 12 to 24 hours has been used.

Adverse Effects

The administration of zonisamide to animals has been infrequent; therefore, there is not a full appreciation for the incidence of adverse effects. In clinical studies, the adverse effects reported were sedation (transient), ataxia, and vomiting (Dewey et al., 2004). Rare idiosyncratic hepatic injury and renal tubular acidosis have been observed in some dogs. There were also some adverse effects that occurred that are similar to those caused by sulfonamides. Because of structural similarity between zonisamide and sulfonamides, this may be of concern.

In safety studies, dogs tolerated doses of 10–175 mg/kg for 52 weeks (pharmaceutical company data). At doses of 1,000 mg/kg to dogs, there was a decrease of locomotor movement, ataxia, and vomiting (pharmaceutical company data). In a pharmacokinetic and safety study (Boothe and Perkins, 2008), clinical laboratory tests remained normal after 8 weeks, but thyroxine concentrations (T_4) may be lower in some dogs. (Sulfonamide-like drugs are known to affect thyroxine synthesis in dogs.)

In one study in cats, safety was evaluated after 9 weeks of daily administration of 20 mg/kg (Hasegawa et al., 2008). Lethargy, anorexia and gastrointestinal problems were observed. Mild gastrointestinal problems observed in half of the cats. Some cats had neurological reactions at high doses.

Bromide (Potassium and Sodium Bromide)

Bromide is the oldest, yet most chemically simple, of the anticonvulsants. It was used in people as early as the mid-1800s and its use was first described in dogs as early as 1907. Because it is toxic in people, it was replaced by other anticonvulsants after the introduction of phenobarbital in 1918. There are no commercial formulations available in the USA, but some are approved in other countries. In the USA, the dose formulations used in animals are prepared by compounding from the active chemical substance. It is now used commonly in dogs, but less common in cats (Podell and Fenner, 1994; Trepanier, 1995). The exact mechanism of action is uncertain, but it appears to stabilize neuronal cell membranes. It may enter the chloride channel and reach intracellular levels in the postsynaptic membrane to hyperpolarizes the neuronal cell membrane.

Pharmacokinetics

Oral absorption of the compounded products is approximately 46%. The volume of distribution (0.45 l/kg) suggests equal distribution in body water. The half-life in dogs is approximately 25 days, but can be variable. Elimination rate can vary depending on the patient's diet (see Section Effect of Diet). Because the half-life is long, chronic daily administration will be necessary for this drug to accumulate to steady-state plasma concentrations. However, during the accumulation phase (loading dose period) the half-life is shorter (15 days; March et al., 2002) which results in faster accumulation. The half-life in cats appears to be a bit less than dogs – about 14 days, and perhaps as short as 10 days (Boothe et al., 2002). Bromide is entirely excreted by the kidneys; therefore, liver impairment or drugs that affect cytochrome P450 enzymes will not affect clearance.

Clinical Use in Dogs

Bromide has been used in combination with phenobarbital to treat refractory seizures, and also has been used as monotherapy (single drug). It is not as effective as monotherapy for treating epilepsy in dogs as phenobarbital (Boothe et al., 2012), but can be acceptable in some dogs. When bromide is administered as a single drug, bromide doses may need to be higher compared to administering bromide with phenobarbital. When used with phenobarbital, phenobarbital doses can be decreased by as much as one-half. One study reported that, in 19% of dogs receiving phenobarbital and bromide, eventually the phenobarbital was discontinued while still maintaining seizure control (Trepanier et al., 1998).

The ACVIM consensus panel (Podell et al., 2016) gave bromide a moderate rating for both monotherapy and add-on treatment. Most often, bromide is used as add-on therapy in refractory epileptics who are receiving other anticonvulsant medications. When potassium bromide (KBr) has been used to treat canine epileptics that have not responded to phenobarbital alone, success rates have been 60% and one author reported as high as 72% improvement in seizure control (Trepanier et al., 1998). After several years of using bromide for treating epilepsy in dogs, a comprehensive review (Baird-Heinz et al., 2012) concluded that "Potassium bromide is not an appropriate therapeutic choice for every dog…" The authors recognized that it can be helpful for some dogs, but also emphasized the importance of careful dosing, monitoring of serum concentrations, and observation for adverse effects to ensure safe and effective therapy.

Maintenance doses: Usually the potassium salt of bromide is used, but sodium bromide also can be substituted. Most veterinary compounding pharmacies have recipes for preparing an oral solution and it is relatively inexpensive. Compounded forms have been tested and shown to be stable for several months. However, adherence to good compounding practices is important to ensure safe and effective dosing. The typical dosage of potassium bromide for dogs is 30–40 mg/kg, orally, once per day. (This translates to 20–27 mg/kg of bromide once per day.) The lower doses are more common when used with phenobarbital, while the higher doses are given when monotherapy is used.

There is high variability among animals with respect to absorption and excretion. Therefore, it is suggested that each patient be monitored for signs of toxicity (ataxia, CNS depression), as well as plasma concentration monitoring, and the dose adjusted if necessary.

Loading doses: Loading doses of 600 mg/kg orally over 3–4 days have been administered in patients that need therapeutic concentrations quickly in the range of 1.0–1.5 mg/ml. If dogs are receiving bromide alone (without phenobarbital), use a starting dose of 40–50 mg/kg/day. In one study, March and colleagues (2002) administered loading doses of 30 mg/kg twice daily for 115 days to achieve steady-state concentrations in the range of 200–300 mg/dl.

In some cases it has been necessary to administer sodium bromide IV as a loading dose. This has been accomplished by mixing sodium bromide (potassium bromide may be responsible for unsafe potassium concentrations in some patients if administered IV), in a solution to deliver an initial dose of 600–1,200 mg/kg over approximately 8 hours. If the plasma concentration is still in the low range, an additional IV loading dose can be administered.

Effect of Diet

Diets high in chloride will cause bromide to be excreted more rapidly (as much as 50% decrease in half-life with high chloride diets); therefore, the diet should be kept constant throughout therapy, or monitor serum bromide concentrations each time the diet is changed. Some prescription diets have either high or restricted chloride content and patients receiving these diets may need dose adjustments.

Clinical Use in Cats

A report and accompanying review evaluated use of bromide in cats (Boothe et al., 2002). In that report, the authors identified a half-life in cats of only 11.2 days. An evaluation of the clinical use showed that there was inadequate seizure control in approximately half of the cats, despite serum concentrations that are reported to be effective in dogs. The most common dose was 30 mg/kg per day. More importantly, approximately half of the cats developed adverse reactions. The most common adverse effect was coughing (see Section Side Effects and Adverse Effects), and follow-up case reports showing bromide-associated lower airway disease in cats concluded that "using bromide for seizure control in cats cannot be recommended" (Bertolani et al., 2012).

Therapeutic Monitoring

Plasma concentrations can be measured at most veterinary diagnostic laboratories. It is a relatively easy measurement in plasma, usually based on the gold chloride reaction and observed color reaction. Ordinarily, effective concentrations are reported as between 1 and 2 mg/ml (100–200 mg/dl). If concentrations are less than 1.0 mg/ml, one should increase the dose. If bromide is used as the sole anticonvulsant (without phenobarbital) concentrations as high as 2–3 mg/ml (200–300 mg/dl) may be necessary.

Side Effects and Adverse Effects

Side effects of polyphagia, polyuria/polydipsia, and behavior changes have been reported in some dogs. Bromide toxicosis (bromism) has been reported with high serum concentrations and/or high doses (Rossmeisl and Inzana, 2009). The signs of toxicosis are largely related to CNS depression and include depression, weakness, ataxia, and decreased proprioception. These signs can be alleviated with a decrease in dose, or diuresis to increase renal elimination. The authors of this study (Rossmeisl and Inzana, 2009) indicated that the most common reason for development of bromism was suboptimal monitoring of serum concentrations that resulted in concentrations that were too high. They also indicated that mistakes during compounding could result in dosing errors. Bromide can cause some stomach upset in some dogs, and administration with food may alleviate this problem. There is some evidence that bromide treatment, or the combination of phenobarbital and bromide can increase the risk of pancreatitis in dogs, but clinical confirmation of this risk has not been shown (Steiner et al., 2008). A unique sign of high bromide concentrations is joint stiffness in the rear limbs. Some animals may show signs of sedation for the first 3 weeks of therapy, but develop tolerance with chronic treatment.

In cats, respiratory signs resembling feline asthma (coughing) were identified (Boothe et al., 2002). The mechanism is not known, but it is not believed to be an allergic reaction, but rather a result of airway inflammation. A follow-up case series reported bromide-associated lower airway disease in cats treated with bromide (Bertolani et al., 2012). In some cats there was neutrophilic and eosinophilic inflammation of the airways. The authors concluded that bromide may induce severe and irreversible lower airway disease in some cases, and complete resolution of clinical signs did not occur until bromide therapy was discontinued.

Drug Interactions

When an animal is receiving potassium bromide therapy, it may cause a falsely elevated chloride serum measurement with some assays.

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18

Drugs Affecting Animal Behavior

Margaret E. Gruen, Barbara L. Sherman, and Mark G. Papich

Introduction

Over the past decade, the use of drugs to influence animal behavior has expanded commensurate with that of human behavioral medicine (Crowell-Davis and Murray, 2006; Simpson and Papich, 2003). Drugs, in combination with behavior modification, have been used to manage difficult animal behavior problems. Often these problems are those insufficiently responsive to nonpharmacological approaches alone and may impact the animal's health and welfare. In a general way, drugs may decrease arousal, excitability, and impulsivity and promote behavioral calming. More specifically, behavioral drugs may be used to attenuate repetitive compulsive behaviors, modulate aggression, and help manage organic states (Stein et al., 1994). Psychotropic drugs may be used to decrease the latency to response to behavioral treatment (King et al., 2000b).

The use of behavioral drugs to reduce fears and anxieties may enhance animal welfare and promote safe and humane handling. Extrapolating from the reports of humans who suffer from anxiety disorders and from our direct observations, highly anxious or fearful animals suffer as well and should be treated, behaviorally and pharmacologically, consistent with our mission as veterinarians to reduce animal suffering. We suggest that, particularly for anxiety-related disorders, pharmacological treatment should be considered as a first, rather than last, resort.

This chapter focuses on commonly used behavioral drugs, their presumed mechanism of action, their side effects and their application to clinical veterinary practice. In addition, some new agents, with potential for use in veterinary behavior, are introduced. Although the activities of behavioral drugs have been elucidated in vitro, our knowledge of their activity in the brain of humans or nonhuman animals remains imperfect. Radioactive labeling, advanced imaging, and other techniques have revealed the remarkable complexity of the brain and the interrelationships of systems previously considered distinct. For example, there is increasing evidence that affective disorders are modulated by neuroactive steroids that, in turn, modulate specific neurotransmitters, including those described in this chapter (Eser et al., 2006). In spite of recent expansion in our understanding and application of behavioral drugs, behavioral pharmacology remains in its infancy due to the complexity and multidimensional aspects of behavior.

With few exceptions, the drugs discussed herein are approved for use in humans for the treatment of behavioral disorders; their use in animals is extralabel (Simpson and Voith, 1997). Without an approved claim, it has fallen upon veterinary behaviorists, pharmacologists, and other specialists to examine the relevant data and conduct studies to examine the published record on these medications to predict clinical use and response. One of the greatest challenges in evaluating the studies published in human medicine or from laboratory animal studies is to interpret the data in light of the various species differences that exist in pharmacokinetics, drug metabolism, receptor sensitivity, and susceptibility to toxicosis.

Pharmacokinetic Issues for Behavior Drugs

Pharmacokinetics is covered in more detail in this book in earlier chapters (Chapters 2 and 3). Pharmacokinetics is the science that describes the effect of the body on a drug and a description of the absorption, distribution, metabolism, and elimination (Janicak et al., 2011). In this section are some comments regarding the importance of pharmacokinetic issues to behavior drug therapy. When there is a relationship of the plasma or serum drug concentrations to clinical effect, pharmacokinetics can be helpful to predict pharmacological response. This becomes particularly important when we have few controlled clinical trials of drugs in animals, but comparative pharmacokinetic data.

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Absorption, Metabolism, Clearance, and Distribution

Bioavailability of a drug depends on both the extent and rate of drug absorption. Since most behavior drugs discussed in this chapter are administered orally, absorption becomes a critical pharmacokinetic parameter. Drug absorption is determined by examining the relative concentrations in plasma or serum. These concentrations serve as a surrogate to predict clinical response because the availability of a drug to the central nervous system cannot be easily measured.

Drugs administered orally can be absorbed quickly and avoid significant metabolism, be poorly absorbed because of unfavorable dissolution or solubility, or be absorbed from the gastrointestinal tract (GIT) and then undergo first-pass metabolism, which is the process of intestinal or hepatic metabolism prior to reaching systemic circulation.

Many of the behavior drugs to be discussed in this article are weak bases, for example, the substituted amines that are the tricyclic antidepressants and other centrally acting drugs. These weak bases generally have good lipophilicity, but poor water solubility. However, most are formulated as water-soluble salts (hydrochloride salts of clomipramine, fluoxetine, buspirone). This allows for more rapid dissolution in the GIT, followed by good permeability in the intestine. Subsequently, the oral absorption of most of these drugs is good. However, these drugs – being lipophilic – are also subject to enzyme metabolism in the intestine and liver. For some drugs, extensive intestinal and hepatic metabolism may render these drugs susceptible to the first-pass metabolic effects, which reduces the overall systemic availability.

Drug metabolism is the process whereby drugs are metabolized to active and inactive metabolites or an inactive drug can be metabolized to an active drug (if administered as a prodrug). For the drugs discussed in this chapter, the metabolic fate is determined primarily by hepatic and intestinal metabolism. To our knowledge, there are few drugs used in behavior therapy that are affected much by renal clearance, although the kidneys may be the ultimate route of elimination for conjugated water-soluble metabolites.

Many behavioral drugs are substrates for, or affect, cytochrome P450 (CYP) enzymes, which are microsomal drug-metabolizing enzymes (DeVane, 1999; Janicak et al., 2011) located primarily in the liver and GIT. These enzymes have potential for important pharmacokinetic drug–drug interactions. They are designated by family, subfamily, and isoforms by a number and letter sequence (Tables 18.1 and 18.2). In humans the important CYP enzymes include CYP1A2, CYP2C9-10, CYP2C19, CYP2D6, and CYP3A3/4. The enzymes CYP3A3/4 and CYP2D6 are responsible in humans for 50% and 30%, respectively, of known oxidative drug metabolism. Because these enzymes can be both induced and inhibited by certain drugs, such as those classified as antidepressants, concentrations of other drugs, metabolized by the same CYP 450 enzymes, will increase. For example, in humans fluoxetine and paroxetine inhibit CYP2D6, important in the oxidative metabolism of the tricyclic antidepressants (TCAs). When fluoxetine or paroxetine is used in combination with a TCA, a significant increase of the TCA plasma concentration occurs, potentially causing toxicity unless the TCA dose is reduced (Janicak et al., 1997).

One of the problems in veterinary medicine is that the enzymes, and subsequently their substrates and inhibitors, are not as well characterized as in human medicine (Chauret et al., 1997). The enzyme responsible for the greatest proportion of metabolism in humans is CYP3A4 oxidase. There are only low levels of CYP3A4 in dogs and cats, but other enzymes play a larger role (for example, CYP3A12) (Kuroha et al., 2002). Other enzymes present in dogs are the 1A, 2B, 2C, 3A, and 2D families and subfamilies (Chauret et al., 1997; Kuroha et al., 2002; Court, 2013). There are large interspecies differences in the P450-mediated metabolism in dog and cat microsomes compared to human. The variation is in the metabolic activity, as well as the effect of specific inhibitors on P450 enzyme activity (Chauret et al., 1997). Information on the inhibitory activity of drugs on various enzyme systems in humans should not be broadly extrapolated to dogs and cats (Kuroha et al., 2002).

The other step in drug metabolism is a biosynthetic reaction called conjugation. Drug metabolic conjugation is the process whereby the drug or metabolite is linked with endogenous compounds such as amino acids, glucuronic acid, sulfate, glutathione, or acetyl (acetate). These polar conjugates are more water soluble and more easily excreted than the parent compound. The conjugated products are usually inactive, but there are exceptions.

Just as with the other metabolic reactions, there are tremendous species differences in the conjugation reactions. Dogs lack the ability to acetylate drugs such as sulfonamides; cats have a deficient ability to form glucuronide metabolites with drugs such as salicylate and phenols (such as with acetaminophen metabolites).

The rate of hepatic metabolism is measured by hepatic clearance. Clearance is one of the determinants of elimination half-life (T1/2), the time needed for plasma drug concentrations to decrease by 50% (Janicak et al., 2011). Drugs with short elimination half-lives must be given more frequently to maintain a consistent plasma concentration. With repeated dosing, these drugs also achieve steady-state more quickly. Usually after five half-lives, a drug reaches steady-state, the plasma concentration achieved as long as the dosing schedule or other metabolic processes remain constant. Once plasma

Table 18.1 Psychotropic agents used in dogs

Drug class	Drug name	Dose in dogs	References
α_2 agonist	Clonidine	0.007 – 0.049 mg/kg PRN or q 12–24 h	Ogata and Dodman, 2011
α_2 agonist	Detomidine	0.35 mg/m^2 OTM	Hopfensperger et al., 2013
α_2 agonist	Dexmedetomidine	Dogs: 125 μg/m^2 OTM	FDA, 2015
		May re-dose q 2–3 h	Slingsby et al., 2009
		Cats: 40 μg/kg OTM, IM	
Benzodiazepine (BZD)	Diazepam	0.55–2.2 mg/kg PRN	Papich, 2016
BZD	Alprazolam	0.02–0.1 mg/kg q 8–12 h	Landsberg et al., 2013
		0.02 mg/kg PRN (w/clomipramine)	Crowell-Davis et al., 2003
BZD	Clorazepate	2 mg/kg q 12 h	Papich, 2016
			Forrester et al., 1990
BZD	Lorazepam	0.02–0.1 mg/kg q 8–24 h	Mills and Simpson, 2002
BZD	Oxazepam	0.2–1.0 mg/kg q 12–24 h	Landsberg et al., 2013
Azapirone	Buspirone	2.5–10 mg/dog q 12–24 h or	Papich, 2016
		1.0–2.0 mg/kg q 12 h	
Tricyclic antidepressant (TCA)	Amitriptyline	2.2–4.4 mg/kg q 12–24 h	Juarbe-Diaz, 1997a,b
		2 mg/kg q 24 h	Takeuchi et al., 2000
		0.74–2.5 mg/kg q 12 h	Reich et al., 2000
		1–2 mg/kg q 12–24 h	Papich, 2016
TCA	Clomipramine	1–3 mg/kg q 12 h	Papich, 2016
		1–2 mg/kg q 12 h	King et al., 2000b
		1–2 mg/kg q 12 h	Moon-Fanelli and Dodman, 1998
		1–2 mg/kg q 12 h	
		3 mg/kg q 12 h	Seksel and Lindeman, 2001
		3 mg/kg q 24 h	Hewson and Luescher, 1998a,b
			Rapoport et al., 1992
TCA	Imipramine	2–4 mg/kg q 12–24 h	Papich, 2016
Selective serotonin reuptake inhibitor (SSRI)	Fluoxetine	Start 0.5 mg/kg q 24 h, increase to 1.0 mg/kg q 24 h	Papich, 2016 Dodman et al., 1996a
		1 mg/kg q 24 h	Rapoport et al., 1992
		0.96 mg/kg q 24 h	Wynchank and Berk, 1998a,b
		20 mg/dog q 24 h	
SSRI	Paroxetine	0.5–1 mg/kg q 24 h	Papich, 2016
SSRI	Sertraline	3.42 mg/kg q 24 h	Rapoport et al., 1992
		2.5 mg/kg q 24 h	N. Dodman, Pers. Comm. 2000
			Larson and Summers, 2001
Monoamine oxidase inhibitor (MAOI)	Selegiline	0.5–1.0 mg/kg q am	Calves, 2000
Atypical antidepressant	Trazodone	2–5 mg/kg q 12 h and/or bolus 1+ hour prior to an anxiety-inducing event	Gruen and Sherman, 2008; Simpson and Papich, 2003 Gruen et al., 2014
		To facilitate postsurgical calming	
	Mirtazapine	Small dogs: 3.75 mg q 24 h	Quimby and Lunn, 2013
		20–35 pounds dogs: 7.5 mg q 24 h	
		40–50 pounds dogs: 15 mg q 24 h	
		>75 pounds: 22.5 mg q 24 h	
		>100 pounds: 30 mg q 24 h	
		Cats: 1.88 mg per cat once a day or once every other day	
Anticonvulsant	Phenobarbital	0.45 mg/kg q 24 h	Crowell-Davis et al., 1989
		1.5–2.0 mg/kg q 12 h	Dodman et al., 1992
		5 mg/kg q 12 h (with clorazepate)	Forrester et al., 1993 Papich, 2016
		2–8 mg/kg q 12 h	
	Carbamazepine	4–8 mg/kg q 12 h	Holland, 1988; Haug, 2008
	Gabapentin	10 mg/kg q 8–12 h	

(*Continued*)

Table 18.1 (*Continued*)

Drug class	Drug name	Dose in dogs	References
β antagonist	Propranolol	2–3 mg/kg q 12 h (w/phenobarbital)	Walker et al., 1997
Narcotic antagonist	Naltrexone	2.2 mg/kg q 12–24 h 2.2 mg/kg q 12 h	White, 1990 Papich, 2016
Progestogen hormones	Megestrol acetate (see text)	Males: 2 mg/kg q 24 h × 7 days, then if improved 1 mg/kg × 14 days 2.2 mg/kg q 24 h × 14 days, then 1.1 mg/kg q 24 h × 14 days, then 0.5 mg/kg q 24 h × 14 days 2–4 mg/kg q 24 h × 8 days, reduce for maintenance	Joby et al., 1984 Borchelt and Voith, 1986 Papich, 2016
Hormone	Melatonin	0.1 mg/kg q 8–24 h (w/amitriptyline)	Aronson, 1999

See text for special considerations and side effects.
All doses are per os unless otherwise noted

steady-state is achieved, drug concentration in other tissues, such as the brain, is at equilibrium. The time to reach steady-state is relevant to the use of behavior drugs. Some drugs, such as diazepam in the dog, have short half-lives (T1/2 less than 1 hour) (Papich and Alcorn, 1995). Unless administered more frequently than once every five half-lives, these drugs will never reach a steady-state. On the other hand, drugs with long half-lives will accumulate with chronic dosing and attain steady-state in approximately five half-lives. But, if the half-life is 24 hours or longer, several days may be necessary before the drug accumulates to a level high enough to produce a consistent clinical response. This may be one reason that some antidepressant drugs do not have immediate effects when administered chronically in animals.

The physiological distribution of drugs is determined by their lipid solubility and protein binding. The higher the lipophilicity, the greater is the ability to distribute across biological lipid membranes, as long as plasma protein binding is not so high as to limit the diffusion. Because most behavior drugs are weak bases, protein binding is expected to be low for these drugs. However, this is only assumed, because there is little or no published data documenting the true plasma protein binding for these drugs in dogs and cats. Tissue protein binding, or intracellular trapping of drugs, can increase the distribution of drugs from the plasma to tissue compartment. Most of the behavior drugs are unionized and lipophilic at physiological pH. Some may be trapped in the brain or CSF owing to pH partitioning because these spaces are relatively more acidic than the plasma. Because the tissue concentrations may be high relative to plasma concentrations of these drugs, the apparent volume of distribution for this group of drugs is usually greater than 1.0 l/kg.

Relevant to the behavior drugs is the distribution across the blood–brain barrier (BBB). The BBB consists of unfenestrated capillaries, with tight junctions that prevent large or poorly lipophilic molecules from passing from the blood to the brain (Pardridge, 1999; Jolliet-Riant and Tillement, 1999). There is also a blood–CSF barrier, but it makes up a relatively smaller component to the distribution of drugs to the central nervous system. The BBB also is comprised of transmembrane pumps that effectively transport drugs (and probably other compounds) from the brain back to the blood stream. One of the best known of these transporters is p-glycoprotein. Some drugs are good substrates for p-glycoprotein and other drugs serve as inhibitors of these pumps (Jolliet-Riant and Tillement, 1999).

Neurotransmitters

Details of the autonomic nervous system function and transmission are covered in more detail in Chapters 6, 7, and 8. Presented here are some of the neurotransmitters that are most affected by behavior-modifying drugs. Behavioral drugs act either as stimulators (agonists) or blockers (antagonists) of neurotransmitter receptors, or as inhibitors of associated regulatory enzymes (Baldessarini, 1995; Stahl, 2013). Drugs that modulate naturally occurring neurosignals affect the monoamine neurotransmitters serotonin (5-hydroxytryptamine or 5HT), norepinephrine (NE), and dopamine (DA), as well as acetylcholine (ACh), glutamate, and γ-aminobutyric acid (GABA) receptors, among others. Neurotransmitters have multiple receptor subtypes distributed in specific areas of the body with which they interact. The

Table 18.2 Psychotropic agents used in cats

Drug class	Drug name	Dose in cats	References
Benzodiazepine (BZD)	Diazepam	1–4 mg/cat q 12–24 h 0.2–0.4 mg/kg q 12–24 h 1–2 mg/cat q 12 h	Papich, 2016 Cooper and Hart, 1992
BZD	Alprazolam	0.125–0.25 mg/cat q 12 h	Marder, 1991
BZD	Clorazepate	2 mg/kg q 12 h	Papich, 2016
BZD	Oxazepam	2.5 mg/cat PRN appetite stimulation	Papich, 2016
Azapirone	Buspirone	2.5–5 mg/cat q 12–24 h 5.0 mg/cat q 12 h 2.5–5.0 mg/cat q 8–12 h	Hart et al., 1993 Sawyer et al., 1999 Marder, 1991
Tricyclic antidepressant (TCA)	Amitriptyline	5–10 mg/cat q 24 h 2.5–5.0 mg/cat q 12–24 h 0.5–1.0 mg/kg q 12 h 10 mg/cat q HS	Papich, 2016 Sawyer et al., 1999 Halip et al., 1998 Chew et al., 1998
TCA	Clomipramine	0.5 mg/kg q 24 h 1.25–2.5 mg/cat q 24 h 1–5 mg/cat q 12–24 h	DeHasse, 1997 Sawyer et al., 1999 Papich, 2016
TCA	Imipramine	2–4 mg/kg q 12–24 h	Papich, 2016
Selective serotonin reuptake inhibitor (SSRI)	Fluoxetine	0.5–4.0 mg/cat q 24 h 1 mg/kg q 24 h 1–1.5 mg/cat q 24 h 2 mg/cat q 24–72 h	Papich, 2016 Pryor et al., 2001 Hartmann, 1995 Romatowski, 1998
SSRI	Paroxetine	1.25–2.5 mg/cat q 24 h	Papich, 2016
Monoamine oxidase inhibitor (MAOI)	Selegiline	0.5 mg/kg q 24 h	
Atypical antidepressant	Mirtazapine	1/8–1/4 × 15-mg tablet (1.87–3.75 mg)/cat q 72 h	
	Trazodone	50 mg/cat PRN	Orlando et al., 2016 Stevens et al., 2016
Anticonvulsant	Carbamazepine	25 mg q 12 h	Schwartz, 1994
	Megestrol acetate (see text)	2.5–5 mg/cat q 24 h × 7d, then 5 mg 1–24 × 4/week 5 mg/cat q 24 h × 7–10 days, then 5 mg EOD × 14 days, then 5 mg 2 ×/week; 2 mg/kg q 24 h × 5 days, then 1 mg/kg q 24 h × 5 days, then 0.5 mg/kg q 24 h × 5 days	Papich, 2016 Hart, 1980 Romatowski, 1989

See text for special considerations and side effects. All doses are per os.
q HS, at bedtime.

most selective drugs mimic the natural neurotransmitter's action at only one receptor subtype. Other substances, such as circulating hormones, pituitary peptides, opioid peptides, and neurokinins can also affect behavior (Stahl, 2013).

At the cellular level, neurotransmission alters the function of postsynaptic target neurons. This process, in turn, affects gene expression (Stahl, 2013). The neurotransmitter released from the presynaptic neuron is considered the first messenger. It binds to its postsynaptic receptor and the bound neurotransmitter regulates a second messenger inside the cell of the postsynaptic neuron. This second messenger, in turn, forms transcription factors that, when activated, bind to regulatory regions of genes. This process activates RNA polymerase and the gene is transcribed into its mRNA, leading to translation of the corresponding protein. The protein can influence cellular processes that modulate behavior. Because multiple neurotransmitters are involved in CNS function, each working through multiple receptors, chemical signaling provides the features of both selectivity and amplification.

Discussed below are neurotransmitters or regulatory enzymes known to influence behavior and be affected by commonly used behavioral drugs. The monoamine neurotransmitters include norepinephrine, dopamine, and serotonin. It is now known that many neurons respond to more than one neurotransmitter, a process called *cotransmission* (Stahl, 2013). This may explain why multiple drugs in combination may be particularly effective and why some beneficial drugs act on more than one neurotransmitter. At this time, there is no rational treatment approach based on cotransmission. However, a strategic multiple drug program may enhance treatment success in the future.

Norepinephrine

Norepinephrine (NE) is derived from the amino acid tyrosine, which is transported from the blood and into each noradrenergic neuron by means of an active transport pump (Stahl, 2013). There, tyrosine is acted upon by three enzymes, eventually converting it to dopamine, and then to NE, which is stored in vesicles. NE can be broken down by monoamine oxidase (MAO), located in mitochondria, and catechol-*O*-methyl transferase (COMT), located outside the presynaptic nerve terminal. There are three postsynaptic receptors for NE that are important for the action of behavior-modifying drugs: β_1, α_1, and α_2. Norepinephrine has little activity on β_2 receptors. α_2 receptors are also found presynaptically. Called autoreceptors, they regulate NE release via a negative feedback system.

Most cell bodies for noradrenergic neurons are located in the locus coeruleus area of the brainstem. This region determines whether attention is focused on the external environment (as in response to a threat) or to internal signals (such as pain). There are many specific noradrenergic pathways in the brain, controlling both psychological and physiological activities. For example, projections from the locus coeruleus to the limbic cortex regulate emotions; projections in cardiovascular centers may control blood pressure.

Dopamine

Like norepinephrine, dopamine is synthesized intraneuronally from the amino acid tyrosine. Dopamine (DA) neurons lack the third enzyme that leads to conversion to norepinephrine. The same enzymes that break down norepinephrine (MAO and COMT) break down DA. There are at least five DA receptor subtypes. Best known is the DA_2 receptor, which is stimulated by dopaminergic agonists for the treatment of Parkinson's disease in humans and blocked by DA antagonist antipsychotics. Acepromazine, which is known to most veterinarians, is a well-known dopamine antagonist. Although DA_1, DA_3, and DA_4 receptors respond to antipsychotics, it is not clear to what extent they contribute to the behavioral effects of these drugs. When dopamine receptors are blocked, as with an antipsychotic drug, acetylcholine activity increases. This is because dopamine normally suppresses acetylcholine activity. An increase in acetylcholine activity can lead to extrapyramidal signs, discussed in Section Acetylcholine.

Serotonin

Serotonin, is also called 5-hydroxytryptomine or 5HT. The chemistry and pharmacology of serotonin and other transmitters are covered in more detail in Chapter 19. Abnormalities in central serotonin function have been hypothesized to underlie disturbances in mood, anxiety, satiety, cognition, aggression, and sexual drives (Tollefson and Rosenbaum, 1998). Drugs that enhance serotonin are among the most effective modulators of behavior (Simpson and Simpson, 1996a). Abnormalities in serotonin production or metabolism may underlie some behavior problems in companion animals. For example, dogs that exhibit affective (dominance type) aggression have significantly lower levels of serotonin metabolites in their cerebrospinal fluid than nonaggressive control dogs (Reisner et al., 1996). Canine compulsive disorder may be linked to 5HT dysfunction, based on the responsiveness of dogs that exhibit repetitive spinning, object licking, or light chasing to treatment with drugs that inhibit serotonin reuptake (Hewson et al., 1998a; Luescher, 2003).

For synthesis of serotonin, the amino acid tryptophan is transported into the brain from plasma. Two enzymes are involved in the conversion of tryptophan to 5HT. Analogous enzymes, transport pumps, and receptors exist in the 5HT neuron. Classification of 5HT receptors was reviewed thoroughly by Hoyer and colleagues (1994). There are two key presynaptic receptors, $5HT_{1A}$ and $5HT_{1D}$, and at least six postsynaptic receptors, $5HT_{1A}$, $5HT_{1D}$, $5HT_{2A}$, $5HT_{2C}$, $5HT_3$, and $5HT_4$ (Hoyer et al., 1994). As with NE and DA, presynaptic receptors act as autoreceptors that detect high concentrations of 5HT, inhibit further 5HT release, and slow 5HT neuronal impulse flow. Postsynaptic 5HT receptors regulate 5HT release from the presynaptic nerve ending. The $5HT_{2A}$, $5HT_{2C}$, and $5HT_3$ receptors are implicated in several serotonin pathways in the CNS (Hoyer et al., 1994). Although some $5HT_4$ receptors are in the CNS, their action is primarily localized to the gastrointestinal tract. Additional roles of this receptor on gastrointestinal function is discussed with specific drugs in Chapter 46. Serotonergic nuclei are localized to the raphe nucleus of the brainstem (Stahl, 2013). This area has projections to the frontal cortex, which may regulate mood; the basal ganglia, which may control movement and compulsive behaviors; and the limbic area, which may be involved in anxiety and panic.

There is evidence that the serotonin system may exert "tonic inhibition" on the central dopaminergic system (Tollefson and Rosenbaum, 1998). This may explain the occasional unexpected occurrence of extrapyramidal side effects during therapy with a selective serotonin reuptake inhibitor (Tollefson and Rosenbaum, 1998).

Acetylcholine

Acetylcholine (ACh) is formed in cholinergic neurons from two precursors: choline, derived from dietary sources, and acetyl coenzyme A, which is synthesized in the neuron. There are two major types of cholinergic receptors: nicotinic and muscarinic. Each of these is further divided into numerous receptor subtypes. There are five muscarinic receptor subtypes, M_1 through M_5. M_1 receptors are found at ganglia. M_3 and M_4 are found on smooth muscle and secretory organs, such as those of the GIT, and all five subtypes are found in the central nervous system. A nonspecific blocker of muscarinic receptors is atropine. One of the side effects of some behavior-modifying drugs (e.g. tricyclic antidepressants) is to block muscarinic receptors, thus producing cardiovascular, gastrointestinal, and other side effects. Additional discussion of the cholinergic nervous system is provided in Chapter 8.

Gamma-Aminobutyric Acid

Gamma amino butyric acid (GABA) is the major inhibitory neurotransmitter in the CNS, localized particularly in the cortex and thalamus (Sheehan and Raj, 2009). GABA is synthesized from the amino acid precursor glutamate. Glutamate participates in multiple metabolic functions. The GABA neuron has a presynaptic transporter similar to those of NE, DA, and 5HT. There are two subtypes of GABA: $GABA_A$ and $GABA_B$. $GABA_A$ subtype receptors are allosterically modulated by benzodiazepine receptors and others. Some of the anticonvulsant drugs that affect the GABA receptor are discussed in Chapter 17.

Major Drug Classes

Historically, behavioral drugs were classified according to their first human clinical application (for example, the antidepressant category), although such categorical descriptions have become functionally obsolete. Most behavioral drugs used in human and veterinary medicine have expanded their use well beyond their original clinical application. Traditionally, drugs are further classified according to their chemical structure and neurochemical activity. Tricyclic antidepressants (referring to a common chemical structure) and selective serotonin reuptake inhibitors (referring to neurochemical activity) are examples of drugs classified by their chemical structure and mechanism of action, respectively. The historic and functional drug classifications are retained here, since they provide a useful framework for our understanding of action and side effects of behavioral drugs. Drugs in the same category share many characteristics, including mechanism of action and common side effects. In addition, many reference sources utilize this traditional and logical categorization.

Antipsychotics

The antipsychotics include a number of structurally dissimilar drugs used in humans to treat psychosis, typified by conditions such as schizophrenia, affective disorder, and psychoses associated with organic mental disorders (Nasrallah and Tandon, 2009). Most veterinarians are familiar with acepromazine, one of the drugs in this class that is approved for veterinary medicine (e.g., Atravet, PromAce, or ACE; sometimes incorrectly called acetylpromazine). Acepromazine and other sedatives in this class are discussed in more detail in Chapter 14. Since the conventional antipsychotics produce neurological side effects, they are sometimes called *neuroleptics*. This term is generally not applied to newer, atypical antipsychotics for which neurological side effects are less likely. Antipsychotics block central dopamine (DA) receptors, particularly of the subtype DA_2. Antipsychotics produce ataraxia, a state of relative indifference to external stimuli (Baldessarini, 1995). Most antipsychotics are metabolized by the CYP 450 enzymes belonging to family 2 and 3; therefore it is possible that drug–drug interactions described for people also would be a concern for animals (Simpson and Papich, 2003).

Except for acepromazine (and Prolixin for horses), antipsychotics are not commonly used in modern veterinary behavioral medicine for a number of reasons. First, small animals are rarely diagnosed with "psychosis", and the anxiolytic properties of antipsychotics in animals are minimal. Second, side effects limit their usefulness. When animals are given traditional antipsychotics at relatively high or repeated doses, they often develop catalepsy, a syndrome with immobility, increased muscle tone, and abnormal postures, although reflexes (including the bite reflex) are preserved. Most veterinarians are familiar with the effects of acepromazine on dogs and cats. Third, spontaneous motor activity, caused by dopamine-receptor blockade in the striatum and inactivation of dopamine neurons in the substantia nigra may result from the administration of phenothiazines to animals (Nasrallah and Tandon, 2009). Finally, other important side effects of antipsychotic drugs, summarized below, can be unacceptable.

Antipsychotics can cause extrapyramidal signs (EPS) because of their effect of inhibiting the action of dopamine. EPS are most likely in older, high-potency antipsychotics such as haloperidol. EPS documented in humans include pseudoparkinsonism (stiffness, tremor, shuffling gait), akathisia (motor restlessness), and acute dystonic reactions (tightening of facial and neck muscles). The involuntary muscle movements of EPS have been confused with seizures. Antipsychotic drugs may also reduce blood pressure and elevate prolactin levels. One subclass of antipsychotics, the phenothiazines, may disinhibit learned responses (Aronson, 1999) and may inhibit the learning processes necessary for behavior modification techniques. It has been reported in several textbooks that this group of drugs, particularly acepromazine, increases the risk of seizures in animals and may actually be contraindicated in animals at risk of seizures. However, clinical studies have shown that acepromazine *does not* increase the risk of seizures in dogs (Tobias et al., 2006; Garner et al., 2004; McConnell et al., 2007).

These EPS should not be confused with another adverse effect of long-term antipsychotic medication called *tardive dyskinesia*. Tardive dyskinesia is characterized by oral–facial, limb, or truncal dyskinesia or twisting postures. The differentiation is that EPS usually occur soon after administration of the drugs, while tardive dyskinesia occurs after prolonged chronic treatment. The other important differentiation is that EPS are believed to be caused by a deficiency of dopamine and tardive dyskinesia is caused by an excess of dopamine, or increased dopamine receptor sensitivity caused by chronic administration. Decreasing the dose or withdrawal of the antipsychotic drug will worsen tardive dyskinesia. To our knowledge, tardive dyskinesia has not been described clinically in veterinary patients, though rodent models have been described.

Historically, one phenothiazine antipsychotic, acepromazine (PromAce, ACE, Atravet), has been used in the management of veterinary behavior problems, such as noise phobia, by reducing animals' general attendance to environmental stimuli and producing sedation. The effectiveness of acepromazine as an oral anxiolytic is often disappointing and causes undesirable side effects (Overall, 1998a). However, its intramuscular use may decrease presurgical patient apprehension and reduce the dose of other drugs used as general anesthetics (Light et al., 1993). It also may reduce the dose of other coadministered anesthetics. Owing to the sedative and extrapyramidal effects, acepromazine is not satisfactory for chronic administration. Other agents, such as the benzodiazepines or antidepressants, are preferred because they are more specific for their antianxiety effects and have fewer side effects. A small proportion of companion animals – especially cats – that are administered acepromazine orally will experience spontaneous motor activity, possibly akathisia.

A unique adverse effect reported in horses is penile prolapse. It is usually of short duration, but can last as long as 4 hours and can lead to paraphimosis. Because acepromazine is not used to modify behavior in horses, and is used primarily as an anesthetic adjunct, more detailed information about this adverse effect is listed in Chapter 14.

Phenothiazines have been used to treat compulsive behaviors not satisfactorily responsive to serotonergic drugs or in combination with serotonergic agents (Goodman et al., 1990). Dopamine has been implicated in some forms of stereotypic behaviors, perhaps because of the effect of serotonin (Kennes et al., 1988). Dopaminergic agents such as apomorphine and amphetamine and the dopamine precursor L-dopa can induce stereotypies in animals (Goodman et al., 1990). Thioridazine has been used in one case of aberrant motor behavior in a dog (Jones, 1987). In 2000, proprietary thioridazine (Mellaril) added a label warning that the drug has been shown to prolong the QTc interval and been associated with arrhythmias and sudden death in humans. It was recommended that thioridazine not be used concurrently with fluvoxamine, fluoxetine, paroxetine, propranolol, pindolol, or any drug that affects the QTc interval of the EKG or the CYP2D6 enzymes.

At this time, there is not sufficient information available to recommend safe dosage regimens for the majority of drugs in this class. The side effects associated with the traditional antipsychotics may not apply to the newer drugs, although immobility and transient loss of conditioned responses may be observed. At present their high cost and lack of published data make them impractical for animal use.

Anxiolytics

The anxiolytic drugs include benzodiazepines, azapirones, barbiturates, and antihistamines. Antidepressants (discussed separately, in Section Antidepressants) also have anxiolytic properties. Discussed here are the benzodiazepine and azapirone classes, as well as a special class, nonbenzodiazepine hypnotics.

Benzodiazepines

The benzodiazepines (BZDs) constitute a large class of drugs with a long history of safe and efficacious use in humans. Examples used in veterinary medicine are shown in Figure 18.1. All drugs in the class act on BZD receptors in the CNS to facilitate $GABA_A$, an inhibitory neurotransmitter. They are not direct inhibitors of the GABA receptor site, but modulate the GABA receptor to produce the desired effects. After binding to the $GABA_A$ receptor, these drugs enhance the GABA-mediated conductance through ionic channels and stabilize excitable membranes. The effects of BZD on behavior may be attributed to potentiation of GABA pathways that act to regulate release of monoamine neurotransmitters in the CNS. Examples of drugs in this class with veterinary application are diazepam, clorazepate, alprazolam, lorazepam, oxazepam, orazepam, and temazepam. Anesthetic uses of benzodiazepines are discussed in more detail with anesthetic agents in Chapter 14. The use

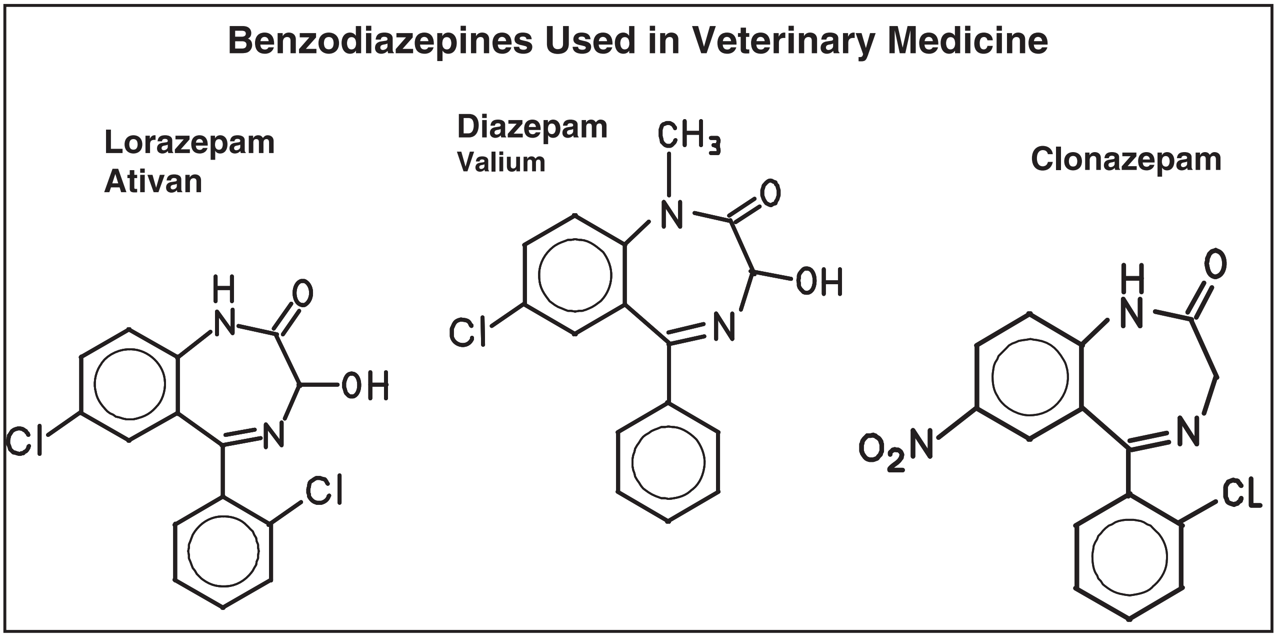

Figure 18.1 Benzodiazepines used in veterinary medicine. Lorazepam (left), diazepam (center), and clonazepam (right).

of benzodiazepines for treating seizures is discussed in Chapter 17 on anticonvulsant drugs.

There are more differences in the pharmacokinetic properties among these drugs than differences that affect the receptor (pharmacodynamic effect). BZDs are used in humans primarily for generalized anxiety disorder or panic disorder (Sheehan and Raj, 2009); they are used similarly in small animals (Simpson and Simpson, 1996b). Dosing schedule can affect pharmacokinetics. For example, diazepam given one time for anxiety will have a lower maximal nordiazepam concentration compared to the same BZD given twice daily (Sheehan and Raj, 2009; Forrester et al., 1990).

After oral administration, behavioral responses to BZDs generally occur within 1 hour, although conflicting studies in animals suggest that the anxiolytic effects are greater after they have been administered for several days (File, 1985). Sedation, ataxia, muscle relaxation, increased appetite, paradoxical excitation, and memory deficits may be observed (Roy-Byrne and Cowley, 1991). Tolerance to sedation, ataxia, and muscle relaxation may develop over the first few days of therapy (Löscher and Frey, 1981). Animals may become ataxic and unstable; therefore, pet owners should be cautioned to assist older animals to avoid falls. Animals should be observed for hyperphagia when given BZDs on a regular basis. Agitation and restlessness may occur as an idiosyncratic response to BZDs. Paradoxic reactions of excitement have been observed in dogs, particularly with administration of alprazolam. If these are observed, the drug should be discontinued and another drug from another class should be selected. Amnesia from BZD has been observed in humans for many years (King, 1992). Also, in animals, memory deficits and diminished conditioned responses may be observed, that is the animal may seem to “forget” what it has been previously taught. Difficulty in learning new behaviors, such as desensitization protocols, may be affected by the memory deficits.

At routine doses, BZDs have little, if any, effect on cardiovascular and respiratory systems (Sheehan and Raj, 2009). BZDs may disinhibit behavior. In humans, manifestations of disinhibition include hostility, aggressiveness, rage reactions, paroxysmal excitement, irritability, and behavioral dyscontrol (Dietch and Jennings, 1988). These effects are also reported in animals (Dodman, 2000), thus benzodiazepines should be used with caution in aggressive animals, particularly dogs, since bite inhibition may be lessened. The potential for behavioral disinhibition serves as a contraindication for the use of benzodiazepines in fearful but aggressive dogs. Bite inhibition may be diminished and the net effect may be an increased, rather than decreased, tendency to bite. Benzodiazepines are controlled substances with the potential for human substance abuse. Pet owners should be screened prior to prescribing and refill requests should be carefully scrutinized.

Although BZDs have a high safety margin, occasionally animals may be exposed to overdoses (accidental ingestion, for example) or have a paradoxical, unexpected reaction. In these instances it may be necessary to reverse the effects. Flumazenil (Romazicon) is a benzodiazepine-receptor antagonist and will inhibit the effects of BZDs. Flumazenil has been used to counteract the adverse effects of large overdoses of BZDs.

After daily administration for more than 1 week, a benzodiazepine should be withdrawn gradually to avoid discontinuation syndrome. Discontinuation syndrome, especially common in high-potency benzodiazepines such as alprazolam, includes nervousness, tremors, or even seizures (Roy-Byrne and Cowley, 1991; Roy-Byrne et al., 1993). The longer a BZD is administered and the higher the dose used, the greater the likelihood of withdrawal reactions when it is discontinued, especially abruptly (Janicak et al., 2011). Signs may be reversed by administration of the BZD. Discontinuation syndrome may be avoided by tapering the BZD dose 25% per week for 1 month. If a discontinuation reaction is observed, signs can be relieved by administering the implicated drug.

BZDs are used in dogs to treat fears and phobias as well as generalized anxiety. Benzodiazepines may be combined with a tricyclic antidepressant such as clomipramine to decrease latency to effect and reduce the panic-like states of thunderstorm phobia (Crowell-Davis et al., 2003) and separation anxiety (Herron et al., 2008). Among cats, BZDs are used for management of urine spraying, travel, and generalized anxiety, such as anxiety associated with changes in a new home environment.

Diazepam

Diazepam (Valium) is the best known of the BZD (Figure 18.1). It has been used for behavior disorders as a sedative, muscle relaxant, anxiolytic, anticonvulsant, and adjunct for anesthesia. Its high lipophilicity and rapid distribution make it suitable for the emergency treatment of seizures because it crosses the blood–brain barrier quickly. Its high lipophilicity also allows it to be absorbed across membranes quickly and is even rapidly and almost completely absorbed from a rectal or nasal administration (Papich and Alcorn, 1995; Musulin et al., 2011). However, the vehicle carrier is not suitable for intramuscular administration.

The pharmacokinetics of diazepam are complex, but have been examined in both dogs and cats (Sheehan and Raj, 2009; Löscher and Frey, 1981; Papich and Alcorn, 1995; Cotler et al., 1984; Musulin et al., 2011). Diazepam undergoes metabolism first to a demethylated metabolite, desmethyldiazepam (also called nordiazepam), and then to oxazepam. Both of these metabolites are active, but not as active or as lipid soluble as diazepam. Desmethyldiazepam is believed to have anticonvulsant properties that are equal to (Randall et al., 1965) or about one-third of (Frey and Löscher, 1982) the potency of diazepam. The pharmacokinetics of diazepam illustrate the tremendous species differences in clearance and elimination. In people, diazepam is considered a drug with low hepatic clearance and a long half-life. The half-life in people is 43 hours, (but may range from 24 to 48 hours) and systemic clearance is 0.38 ml/min/kg. In dogs, the half-life was less than 1 hour, and clearance is in excess of liver blood flow at 57–60 ml/min/kg in one study (Papich and Alcorn, 1995), but had a longer half-life of 5.6 hours and slower clearance (11.5 ml/kg/min) in another study (Musulin et al., 2011). Cats have slower clearance than dogs with the half-life in cats of 5.5 hours and the systemic clearance is 4.7 ml/min/kg (Cotler et al., 1984). In all species, the metabolites of diazepam have longer elimination half-lives than diazepam. For example, the half-life of desmethyldiazepam is 51–120 hours in people, 21.3 hours in cats, and 2.2–2.8 or 6.7 hours for dogs (Papich and Alcorn, 1995; Cotler et al., 1984; Musulin et al., 2011). These differences show that for long-term treatment, diazepam is not suitable for dogs because frequent administration is necessary to avoid high peaks and low troughs. However, its short half-life and ability to attain therapeutic concentrations makes it suitable for short-term use. This large difference in pharmacokinetics among species for diazepam means that information published for diazepam in humans will not apply to dogs. For example, CYP 450 enzyme inhibition and other drug interactions cited for people are not likely in dogs because of the already-high systemic clearance. But, because liver clearance is dependent primarily on hepatic blood flow, changes in hepatic perfusion will drastically affect diazepam clearance. Clearance is expected to be altered in dogs with congenital or acquired hepatic vascular shunts.

As an oral anxiolytic in solid form in dogs, it has a benign taste, and is relatively easy to administer directly or by mixing in moist food. However, particularly for panic-like states of thunderstorm phobia and separation anxiety, clinicians anecdotally report the anxiolytic performance of diazepam to be disappointing. Because hepatic clearance is high in dogs, oral doses are likely to be less effective than IV doses. If used orally in dogs, high doses may be sufficient to produce ataxia but insufficient to reduce anxiety. Other agents, such as alprazolam, may be more satisfactory, or a regime of daily, rather than PRN dosing, may be more effective. Alternatively, rectal or nasal administration of diazepam can be considered (Papich and Alcorn, 1995; Musulin et al., 2011).

In cats, oral diazepam has been used for treatment of urine spraying (Cooper and Hart, 1992; Hart et al., 1993). In open trials, efficacy was approximately 55%, although relapse was common on discontinuation (Marder, 1991; Cooper and Hart, 1992). Concerns about side effects from oral use in cats have decreased its application in veterinary medicine. The most serious adverse reaction associated with diazepam in companion animals is that of idiosyncratic hepatic necrosis in cats. Hepatic necrosis from diazepam is a rare but often-fatal condition, and has been documented in cats given oral diazepam. The specific pathogenic mechanism is not known (Center et al., 1996; Hughes et al., 1996). Diazepam undergoes complex metabolism to intermediate compounds. It is possible that in susceptible cats, an aberrant metabolite is produced that is responsible for the hepatic toxicosis. In the cats reported, the reaction occurred within 7 days of oral administration of generic or proprietary diazepam (Center et al., 1996; Hughes et al., 1996). It is possible that the metabolites responsible for the toxicosis are more likely to be produced from oral administration because of first-pass metabolism, compared to parenteral administration (van Beusekom, 2015). Idiosyncratic hepatic necrosis has not been reported after administration of other oral benzodiazepines, although that negative finding does not eliminate the possibility. However, the adverse event may be less likely with lorazepam and oxazepam, which are conjugated directly without undergoing intermediate metabolism. Alprazolam and temazepam appear to have only one intermediate (alphahydroxy) metabolite before undergoing conjugation. Compared to diazepam, these alternative drugs may be less likely to induce hepatic toxicosis in cats but safety studies are needed to confirm this theory.

Clorazepate

The BZD clorazepate (Tranxene) is metabolized in the acidity of the stomach to its active metabolite, nordiazepam, before absorption (Sheehan and Raj, 2009; Forrester et al., 1993). Clorazepate is used in dogs for treatment of anxiety disorders, particularly thunderstorm/noise phobia. Mean peak nordiazepam levels were detected approximately 98 minutes after a single oral dose of clorazepate, and 153 minutes after multiple oral doses (Forrester et al., 1990). The elimination half-life after a single dose (284 minutes) was not significantly different than after multiple doses (355 minutes) (Forrester et al., 1990). An oral dose of 2 mg/kg q 12 h maintains concentrations of the active metabolite, nordiazepam, in the range considered therapeutic in humans (Forrester et al., 1990). Excessive ataxia and sedation are uncommon (Forrester et al., 1990). Although available in a sustained-delivery formulation, one pharmacokinetic study in dogs found no difference in the serum disposition compared to immediate-release clorazepate (Brown and Forrester, 1991).

Alprazolam

Alprazolam (Xanax) is a high-potency benzodiazepine shown in humans to be an effective treatment for panic disorder (Figure 18.1). Its use has increased since the availability of generic forms. It is used in dogs to treat the panic-like states of separation anxiety, thunderstorm phobia, and other phobias, as well as generalized anxiety. In people, it has a more rapid onset of action and shorter elimination half-life than diazepam, but these comparisons have not been reported for animals. In humans (Sheehan and Raj, 2009; Brandwein, 1993), plasma concentrations vary greatly among patients administered identical doses of alprazolam. As in humans, higher doses of alprazolam may be required for panic-like states in dogs, such as thunderstorm phobia and separation anxiety, compared to general anxiety. Thus, individualized dosing may be required to achieve treatment success with the fewest adverse effects (Sheehan and Raj, 2009). Paradoxical excitement occurs in some canine patients given alprazolam. In such cases, the drug should be discontinued and a drug in another class should be selected. The mechanism of this paradoxical reaction is not known. Canine patients receiving alprazolam at a moderately high dose once a day, as may occur with separation anxiety or thunderstorm phobia, are at risk for withdrawal-induced anxiety or tremors prior to the next day's dose, due to its short elimination half-life (Crowell-Davis and Murray, 2006). This may be avoided by administering the drug twice a day and not skipping doses. To terminate the drug, alprazolam should be withdrawn slowly, decreasing the dose over weeks.

Oxazepam and Lorazepam

As mentioned previously, oxazepam (Serax) and lorazepam (Ativan) are metabolized directly via phase II conjugation to inactive compounds (Sheehan and Raj, 2009). Both oxazepam and lorazepam have been used by veterinarians as sedatives, anxiolytics, and anticonvulsants, but they are not as well-known as diazepam. In cats, oxazepam has been used as an appetite stimulant. There are no active metabolites for either oxazepam or lorazepam. Because conjugation reactions are usually preserved, even when there is hepatic disease, these drugs are recommended for individuals with compromised liver function, for aged canine subjects in which metabolism may be slowed (Sheehan and Raj, 2009), and in cats, in which phase II metabolism may be less likely to trigger idiopathic hepatic necrosis. Lorazepam has the advantage of a greater and more prolonged distribution to the CNS than other BZDs. Lorazepam in healthy dogs has a half-life of 0.88 hours, a systemic clearance less than half that of diazepam at 19.3 ml/min/kg, and oral availability of 60% (Papich, unpublished research). Therefore, as an oral drug it may be a suitable alternative to diazepam.

In dogs, benzodiazepines may be used with tricyclic antidepressants for treatment of thunderstorm phobia (Crowell-Davis et al., 2003) and separation anxiety (Takeuchi et al., 2000). When alprazolam is given concomitantly with fluoxetine in humans, the result is a 30% increase in alprazolam levels (but no significant increases in fluoxetine or norfluoxetine plasma concentrations) due to CYP3A inhibition (Lasher et al., 1991). Therefore, coadministration may permit a lower dose of alprazolam to be effective. Fluvoxamine inhibits the CYP3A4 enzyme and can be associated with increased levels of alprazolam (Sheehan and Raj, 2009). In fact, the use of a BZD and an SSRI is a useful strategy with panic disorder in humans refractory to single drug therapy (Stahl, 2013). Similar strategies may be helpful in animals, particularly dogs. Oxazepam has been shown in humans to decrease turnover of serotonin and norepinephrine (Sheehan and Raj, 2009). In contrast, in one pharmacokinetic study in dogs, clorazepate was used concurrently with phenobarbital (Forrester et al., 1993). The amount of the active metabolite nordiazepam in circulation during each dose interval was significantly reduced compared to administration of clorazepate alone (Forrester et al., 1990).

Azapirones

This class of anxiolytics is represented clinically by one drug, buspirone (Buspar). Buspirone was the first nonsedating, nonbenzodiazepine anxiolytic drug to be developed and marketed (Robinson et al., 2009). Buspirone acts as a full agonist at presynaptic $5HT_{1A}$ receptors, with resulting decrease in serotonin synthesis and inhibition of neuronal firing. It also acts as a partial agonist at postsynaptic $5HT_{1A}$ receptors. In serotonin deficit states, buspirone acts as an agonist (Robinson et al., 2009). Buspirone also has dopaminergic effects.

Buspirone is not a substrate for CYP 450 enzymes, nor does it inhibit them (Robinson et al., 2009). It has no interactions with benzodiazepines and there are no withdrawal concerns after long-term use (Stahl, 2013). In humans, buspirone has been effective for treatment of generalized anxiety disorder, but not for the control of panic disorder. Buspirone has demonstrated efficacy in certain animal models of anxiety, such as the conditioned avoidance response. In dogs, buspirone does not appear to be particularly therapeutic for the panic-like condition of thunderstorm phobia or separation anxiety, but it has been used for generalized anxiety.

Because it has a short elimination half-life, buspirone must be administered two or three times per day. Buspirone has a benign taste and may be given with food. In contrast to benzodiazepines, buspirone produces no sedation, no memory or psychomotor impairment, and no disinhibition phenomenon. Unlike the benzodiazepines, buspirone produces no immediate behavioral effects. Its beneficial effects are not observed until administration for several weeks. Side effects are uncommon and mild, but may be noted immediately. They include gastrointestinal signs and alterations in social behavior (often reported as increased "friendliness"). Buspirone has no potential for human abuse.

In cats, buspirone is used to modulate states of high arousal, including feline urine spraying. In an open trial, improvement was observed in 55% of cats, with a 50% relapse rate following the cessation of treatment (Hart et al., 1993). It has also been used to reduce anxiety in the "pariah" cat in cases of intercat aggression within a household (Overall, 1994a, 1999a).

Buspirone can be used to augment certain antidepressants. If used with an SSRI, increased efficacy is possible if intraneuronal serotonin has been depleted. Buspirone also may act directly on autoreceptors to inhibit neuronal impulse flow, possibly allowing repletion of 5HT stores. Also, buspirone may act at $5HT_{1A}$ receptors to aid in the targeted desensitization of $5HT_{1A}$ autoreceptors (Stahl, 2013). In humans, buspirone may be used to augment SSRI treatment for obsessive–compulsive disorder (OCD) with success in some studies (Janicak et al., 2011) but not others (Grady et al., 1993). In dogs, buspirone has been used with tricyclic antidepressants to treat separation anxiety (Takeuchi et al., 2000) and with an SSRI (fluoxetine) to treat a complex case involving anxiety, aggression, and stereotypic behavior (Overall, 1995).

Nonbenzodiazepine Hypnotics

In cases of acute-onset and severe phobic states, such as thunderstorm phobia and separation anxiety, there is a need for safe and rapid reduction in responsiveness to environmental stimuli and initiation of sleep, with relatively short duration of action and rapid recovery. The unpredictable and often disappointing effect of benzodiazepines and phenothiazines, and the long latency to effect of buspirone, leave a void with regard to such application. Nonbenzodiazepine hypnotics hold some promise, since they are used to facilitate and maintain sleep for 3–7 hours in humans, although no published reports on their clinical use in dogs are available to date. Drugs in this class include zaleplon (Sonata), eszopiclone (Lunesta), and zolpidem (Ambien). These drugs are sometimes referred to as the *Z-drugs* to distinguish their effects from traditional benzodiazepines. Like benzodiazepines, zolpidem potentiates the action of GABA, an inhibitory neurotransmitter. However, it does not cause muscle relaxation or produce anticonvulsant properties. $GABA_A$, a target site of benzodiazepines, has omega-1, -2, and -3 receptors. Benzodiazepines act on all receptors, but zolpidem, and other drugs known as Z-drugs, only act on the omega-1 receptor to produce sedation. Zaleplon has an ultrashort elimination half-life in humans, suggesting it unlikely to be useful in dogs. The newest agent, eszopiclone, is registered for the long-term treatment of insomnia in humans (Brielmaier, 2006), but there are no reports of its clinical use in animals.

Zolpidem is a sedative of the imidazopyridine class. The usual human dose is 5–10 mg (also available as *CR*, controlled release formulation), used to promote and maintain sleep. In a report of 33 cases of accidental ingestion of zolpidem by dogs presented to a poison control center, 40% exhibited signs of depression/sedation and 40% demonstrated signs of hyperactivity (Richardson et al., 2002). Although doses were extreme (up to 21 mg/kg), all dogs recovered. Controlled, single-dose administration of a high (0.5 mg/kg) and low (0.15 mg/kg) dose of zolpidem in dogs showed either no clinical effect (low dose) or paradoxical CNS stimulation followed by mild sedation (high dose) (Giorgi, 2012). Other signs reported in research dogs included vocalization, restlessness, anxiety, dysphoria, rage reaction, excitement, muscle spasticity, and hyperreflexia. These findings suggest that zolpidem is unlikely to be clinically useful in dogs.

Antidepressants

This group comprises perhaps the largest group of agents used to manage behavior problems in small animals. This category of antidepressants includes a number of classes of drugs. Depending on the reference consulted, various classification systems have been used to group these drugs. Following convention, here, the structurally similar tricyclic antidepressants (TCAs) are discussed together. Drugs in this class include amitriptyline, imipramine, doxepin, and clomipramine. The functionally similar selective serotonin reuptake inhibitors (SSRIs) are discussed as a class. Drugs that act to inhibit the enzyme monoamine oxidase (MAOIs) are discussed as a class. Grouped as a separate "atypical" class are those antidepressants that fall outside the aforementioned three groups. This group includes the newer, heterocyclic drugs, such amoxapine, maprotiline, venlafaxine, and mirtazapine, which resemble TCAs, and older drugs, such as trazodone and bupropion, which have distinct chemical structures. The classes described above differ in their mode of action, their side effect profile, and their relative efficacy in certain behavioral disorders.

All antidepressants have important antianxiety properties, and at routine doses are generally well tolerated by animals. To some extent, all share a similar action, which is to alter neurotransmitter concentration (primarily norepinephrine and serotonin) at the receptor sites. The unifying theory that explains their efficacy is the *monoamine theory of depression* described by Schildkraut in 1965 (Maes and Meltazer, 1995), which stated that depression in people is caused by a deficiency of monoamine neurotransmitters. Initially, this theory focused on norepinephrine, but it became clear later that serotonin (5HT) also was an important neurotransmitter. The relationship of the mechanism to the neuronal effects and clinical response is complex. The action affecting reuptake of neurotransmitters occurs rapidly, but the clinical response may take days to weeks for maximum effect. This implies that the increased presence of neurotransmitters norepinephrine and 5HT also may affect the sensitivity of receptors, either presynaptically or postsynaptically.

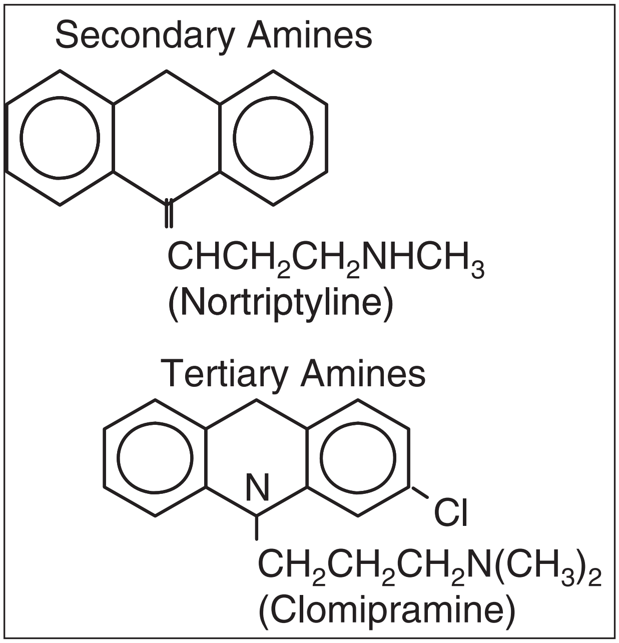

Figure 18.2 Tricyclic antidepressants (TCAs) used to treat behavior disorders in veterinary medicine. Nortriptyline is a secondary amine and clomipramine is a tertiary amine. Most of the other TCAs used in veterinary medicine – amitriptyline (Elavil), doxepin (Sinequan), and imipramine (Tofranil) – are also tertiary amines.

Tricyclic Antidepressants

The tricyclic antidepressants (TCAs) are so called because they have a three-ring nucleus (Figure 18.2). Chemically they resemble phenothiazines, but their actions differ considerably. The TCAs inhibit the reuptake of 5HT and norepinephrine. Specific drugs vary in the extent to which they inhibit one transmitter more than the other. Some of these drugs familiar to veterinarians are human-label drugs that are used off-label. These include the tertiary amines amitriptyline (Elavil), doxepin (Sinequan), and imipramine (Tofranil). The secondary amines, such as nortriptyline (Pamelor) and desipramine (Norpramin), are not as well known to veterinarians. Secondary amines (Figure 18.2) are relatively selective inhibitors of norepinephrine, but the tertiary amines inhibit both serotonin and norepinephrine (Figure 18.3). Clomipramine (Clomicalm, veterinary label; and Anafranil human label), another tertiary amine, is most selective among the TCAs for blocking the reuptake of 5HT. TCAs also have antagonist activity for the α-adrenergic (α_1) receptor, and can produce antihistaminic and anticholinergic effects. The antagonist activity on these receptors is responsible for some of the side effects reported with TCAs, but most likely plays little role in their efficacy. As with other drugs in this chapter, there are species-specific differences in metabolism, elimination, and susceptibility to side effects that will be discussed in more detail with specific drugs.

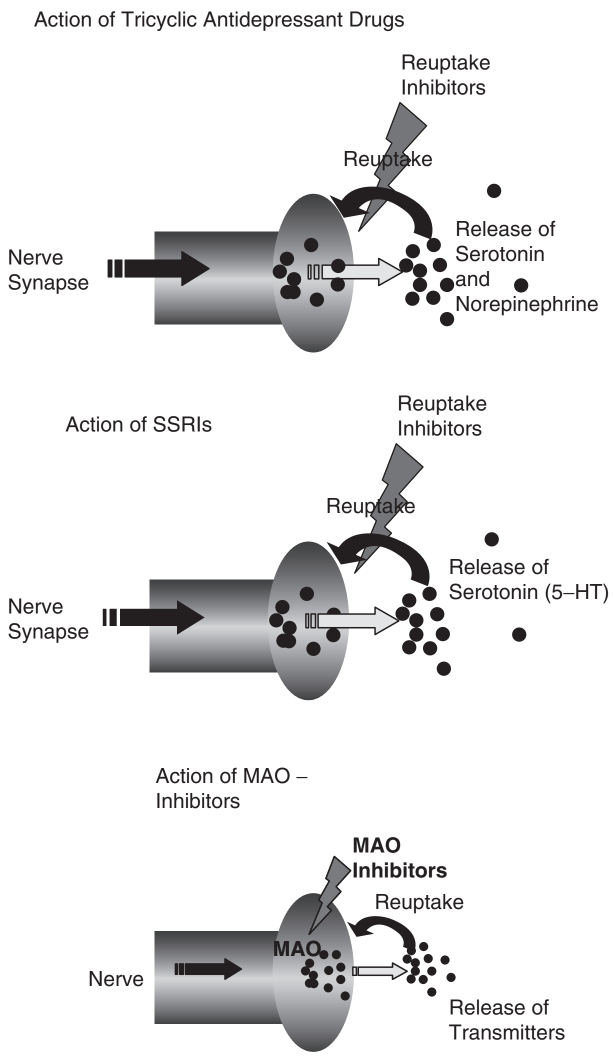

Figure 18.3 Action of drugs used to treat behavior disorders in animals. Tricyclic antidepressants (top panel) inhibit the reuptake of serotonin and norepinephrine. Selective serotonin reuptake inhibitors (SSRI) (middle panel) selectively inhibit reuptake of serotonin without much effect on other receptors. Monoamine oxidase inhibitors (MAO-inhibitors) (bottom panel) inhibit the enzymatic breakdown of monoamines in the presynaptic nerve terminal. Specific types of MAO exist in the nervous system, such as MAO type A and MAO type B, depending on location.

TCAs have a long history of efficacious use in humans, but in recent years, they have been largely replaced by the more specific selective serotonin reuptake inhibitors (SSRI). The low cost, efficacy, and tolerance of the TCAs make them particularly useful in veterinary behavioral therapy. In general, the TCAs modulate excessive arousal and reduce anxiety. TCAs may enhance learning in specific circumstances (Mills and Ledger, 2001). Unlike the benzodiazepines, the TCAs do not disinhibit behavior. TCAs are used in dogs to manage mild aggression, canine compulsive disorders, and various anxiety states (Juarbe-Diaz, 1997a,b). TCAs may be used in cats to control certain forms of aggression, inappropriate urination and spraying, excessive grooming, anxiety states, and excessive vocalization. In horses the TCAs have had limited use and there is little known about pharmacokinetics. Tricyclic antidepressants, such as amitriptyline or imipramine, via their serotonin and norepinephrine reuptake inhibitor effects, may enhance behavioral calming; side effects can include mild sedation and anticholinergic effects in horses. Imipramine has been used to reduce anxiety in breeding stallions and is associated with masturbation and erection in stallions when in a sexual context. At high doses, imipramine (2–4 mg/kg q 24 h, PO) can cause muscle fasciculations, tachycardia, and hyperresponsiveness to sound, likely due to norepinephrine effects.

Pharmacokinetic properties: TCAs are extensively metabolized and are CYP 450 enzyme substrates. In people, coadministration of drugs that inhibit the CYP 450 enzyme that is important for their metabolism can cause a fourfold increase in serum levels of TCAs. Drugs of the selective serotonin reuptake inhibitor class, described below, are potent inhibitors of CYP 450 enzymes (Mealey, 2002). Despite these potential risks, specific reports in the veterinary literature of drug interactions caused by coadministration of other drugs with TCAs are lacking. One reason for the lack of reported problems may be the high systemic clearance compared to humans, which indicates that there is less influence on changes in enzyme activity (discussed in more detail in Section Clomipramine) and the high therapeutic index in animals. Nevertheless, because of the potential for drug–drug interactions in humans, caution is advised when prescribing a TCA with other drugs known to affect drug metabolism. Because of the time to reach pharmacokinetic steady-state, and the time for modulation of the receptors affected by TCAs, therapeutic effects may not be seen for 2–4 weeks. Generally, withdrawal syndromes have not been a problem in animals. Nevertheless, to avoid the possibility of such reactions and to avoid a rebound of the problem being treated, slow discontinuation of any TCA is recommended.

The TCAs have also been used to treat pain syndromes. As reviewed by Micó et al. (2006), there is good evidence that TCAs are effective in people for treating pain, especially neuropathic pain, and may be used in combination with other drugs. Some veterinarians have also used these drugs for treatment of pain, although there are no published reports. The mechanism for analgesia is not entirely understood. It is probably not via the same mechanism that affects anxiety and depression because the analgesic effects have been documented to occur at lower doses and the onset of pain relief occurs faster than relief from depression and anxiety. The proposed mechanism of action for analgesia is via increased availability of norepinephrine and serotonin at the synapse, but other neurotransmitters also could be involved. Amitriptyline seems to be the gold standard for analgesic uses, and other antidepressants, such as SSRIs, do not appear to be as effective. Of note, the drug tramadol (Ultram) used for treatment of pain (discussed in Section Tramadol and in Chapter 13) has some opioid effects, but, in addition, has similar effects to TCAs – inhibition of serotonin and norepinephrine reuptake.

Adverse effects: Adverse effects may occur soon after administration of the drug or after chronic use. Side effects include mild sedation, gastrointestinal side effects – especially vomiting – antihistamine effects, and anticholinergic effects (Nelson, 2009). Anticholinergic effects may include dry mouth (and consequent increased water consumption), constipation, and urinary retention. The use of TCAs is contraindicated in cases of glaucoma or keratoconjunctivitis sicca. Tricyclic antidepressants may also lower seizure threshold and potentiate seizures in predisposed animals (Juarbe-Diaz, 1997a). Agranulocytosis has been associated with TCA administration, but it is rare (Alderman et al., 1993).

A particularly serious adverse effect is possible when TCAs are administered at high doses, as in an accidental ingestion by a pet, for example. High concentrations of TCAs can cause a quinidine-like membrane-stabilizing effect that can lead to fatal cardiac arrhythmias if patients are not treated promptly (Nelson, 2009; Johnson, 1990; Wismer, 2000). If animals receive a high dose (a toxic dose may be over 15 mg/kg), an ECG should be monitored immediately for conduction disturbances. If available, blood pressure should be monitored as well. Treatment of overdoses should be initiated at once, because deaths have occurred within 2 hours. Contact an animal poison control center for specific therapy. Treatment consists of gastric lavage, activated charcoal, a suitable cathartic (*not* containing magnesium), and sodium bicarbonate therapy. Antiarrhythmics that do not affect conduction may be indicated, such as lidocaine, but other class I antiarrhythmics such as procainamide and quinidine are contraindicated (antiarrhythmic drugs are discussed in more detail in Chapter 22).

The cardiotoxic effect described above should not be confused with the effect on the heart produced as an anticholinergic side effect or adrenergic blockade by TCAs. These drugs may elevate heart rate in some individuals caused by the anticholinergic effects, or lower the heart rate in others as a reflex response to the α_1-adrenergic effects. Before administering a TCA to animals, it is suggested to conduct a cardiac assessment (evaluate history, auscultate, ECG if indicated) prior to administration (Pacher et al., 1999). In a study in which effects on the cardiac ECG were evaluated in otherwise healthy dogs administered either clomipramine or amitriptyline, there was no evidence of ECG abnormalities (Reich et al., 2000). In this study, the drugs were administered at doses recommended for treatment of behavior problems.

TCAs may be difficult to administer directly or disguised in food since they have a lingering bitter taste. Biting the tablet can induce taste aversion, future dosing avoidance, and hypersalivation.

Amitriptyline

Amitriptyline is used in humans for depression, anxiety disorders, and for certain types of chronic or neuropathic pain (Anderson, 2001). It exerts active reuptake inhibition on serotonin receptors relative to norepinephrine receptors. It has strong anticholinergic, antihistamine, α_1-adrenergic, and analgesic properties (Anderson, 2001; Nelson, 2009). Nortriptyline, also commercially available (Pamelor), is the active metabolite (Anderson, 2001; Boothe, 2001).

Amitriptyline (Elavil) has been a useful drug in animals to enhance behavioral calming and augment a behavioral treatment program (Anderson, 2001; Hart and Cooper, 1996). In dogs, amitriptyline has been used for treatment of separation anxiety (Takeuchi et al., 2000), aggression (Anderson, 2001; Reich, 1999), and repetitive self-trauma (Overall, 2013, 1998b). The time for maximum effect is 2–4 weeks (Anderson, 2001). In cats, amitriptyline has been used for psychogenic alopecia (Sawyer et al., 1999).

Reported side effects in cats include weight gain, somnolence, and decreased grooming (Chew et al., 1998). Once-a-day, nighttime dosing is recommended to avoid excessive daytime sedation (Chew et al., 1998). Because there is a generic formulation, it has been popular among veterinarians desiring inexpensive treatments for patients.

Among the most common use in cats is administration for urinary disorders. It has been used for urine marking and inappropriate urination secondary to idiopathic interstitial cystitis (Chew et al., 1998). Amitriptyline stimulates β-adrenergic receptors in smooth muscle, including the urinary bladder, to cause a decrease in smooth muscle excitability and an increase in bladder capacity (Anderson, 2001; Chew et al., 1998). It also has been one of the more effective drugs for treating pain syndromes (Micó et al., 2006). For these reasons, it has been used to treat feline interstitial cystitis at an initial dose of 10 mg/cat once in the evening (Chew et al., 1998; Buffington et al., 1999). It is not known if an analgesic effect or behavior-modifying effect plays a role in the efficacy for treatment of this disease.

Imipramine

Imipramine (Tofranil) is a TCA with properties similar to amitriptyline except that imipramine has similar affinity for norepinephrine and serotonin receptors. Imipramine has only moderate affinity for histamine-H_1, or muscarinic (anticholinergic) receptors. Imipramine has more serotonergic activity, fewer anticholinergic effects, and modest α-agonist properties. In humans, imipramine has an elimination half-life of approximately 12 hours. Pharmacokinetics have not been reported for dogs, but empirically it has been administered twice daily to dogs.

Imipramine has been successfully used in humans to treat panic disorder in adults and nocturnal enuresis (bed-wetting) in children. It is a modestly priced treatment for narcolepsy in dogs and horses (Coleman, 1999). Imipramine may be used to treat separation anxiety (Marder, 1991; Overall et al., 2001), particularly in those cases in which urine house-soiling is problematic (Sherman, pers. obs.). Imipramine may also be used in cases of estrogen-dependent urinary incontinence in dogs intolerant to phenylpropanolamine. In one study of laboratory beagles, after 14 days treatment at a high dose (10 mg/kg q 24 h), imipramine improved abnormal "withdrawn and depressed" behavior (Iorio et al., 1983).

Clomipramine

Clomipramine (Clomicalm, veterinary label; Anafranil, human label) has the most serotonergic activity of all the TCAs. It is approved in humans for the treatment of obsessive–compulsive disorder (OCD) and was the first veterinary drug approved by the FDA to treat separation anxiety in dogs.

Dogs metabolize clomipramine more rapidly than humans, which affects clinical use. The average half-life in dogs is 5 hours (King et al., 2000a) to 7.2 hours (Hewson et al., 1998a) after single dose administration, and 2.1–4 hours after repeated doses (King et al., 2000b) The half-life of, desmethylclomipramine, an active metabolite, is 2.9 hours (King et al., 2000a), 1.9 hours (Hewson et al., 1998a), and 2.2–3.8 hours after multiple doses (King et al., 2000b). By comparison, the half-life in humans is 24 hours (Nelson, 2009) to 33 hours, and has been reported to be as high as 36–50 hours (Evans et al., 1980). The systemic clearance in dogs is rapid (23.3 ml/kg/min). The evidence indicates therefore, that in dogs, clomipramine is a high clearance drug for which hepatic blood flow is the most important factor in clearance. Changes in enzyme activity (cytochrome P450 alterations) or protein binding are not expected to affect clomipramine pharmacokinetics as it does in humans.

Oral absorption is only 16–20% in dogs (King et al., 2000a); the low systemic availability is probably reduced because of first-pass hepatic clearance. There are also differences between humans and dogs regarding metabolite profile. In dogs, the ratio of clomipramine to the desmethylclomipramine metabolite is 3 : 1, whereas in people this ratio is only 1 : 2.5. Clomipramine is believed to act primarily as a serotonin reuptake inhibitor, but the desmethyl metabolite is probably most responsible for the anticholinergic side effects. This may explain why there appears to be fewer anticholinergic side effects in dogs compared to people from this drug.

Despite the relatively short half-life in dogs, it has been effective when administered every 12 or 24 hours (King et al., 2000b). Clomipramine has been effective for treatment of canine compulsive disorder (Hewson et al., 1998b; Seksel and Lindeman, 2001) such as tail chasing (Moon-Fanelli and Dodman, 1998), or acral lick granuloma (Goldberger and Rapoport, 1991; Rapoport et al., 1992). Clomipramine (Clomicalm) is approved in the USA for the treatment of separation anxiety in dogs (King et al., 2000b; Simpson, 2000). Although there has been debate concerning the relative merits of behavioral versus pharmacological components to treatment (Podberscek et al., 1999), dogs treated with clomipramine (1–2 mg/kg q 12 h) for separation anxiety and video recorded when left alone showed reduction in anxiety-related behaviors compared to pretreatment control periods (Cannas et al., 2014). In one study, clomipramine was no more effective than controls for the treatment of dominance-related aggression in dogs (White et al., 1999). Clomipramine may be helpful in some cases of noise phobia (Seksel and Lindeman, 2001), but additional treatment with a benzodiazepine, such as alprazolam, may be necessary (Crowell-Davis et al., 2003). When clomipramine compared to placebo was given to dogs every 12 hours for 7 days prior to prior to transport, dogs reduced signs of fear and anxiety and reduced plasma cortisol levels (Frank et al., 2006).

Clomipramine has been used in cats to manage urine spraying (DeHasse, 1997; King et al., 2004), hyperesthesia (Sherman, pers. obs. 2000–2002), and some feline compulsive behavior, including psychogenic alopecia (Sawyer et al., 1999). A study of the pharmacokinetics of clomipramine and its active metabolite desmethylclomipramine after single-dose intravenous and oral administration showed marked pharmacokinetic variability and an average half-life of 12.3 h was calculated for intravenous clomipramine (Lainesse et al., 2006). Another study of single-dose oral clomipramine revealed significant sex-related differences in pharmacokinetics parameters (Lainesse et al., 2007). The results of these studies suggest potential genetic variability in clomipramine metabolism and an explanation for the variability in clinical response observed in cats.

Selective Serotonin Reuptake Inhibitors

As the name implies, selective serotonin reuptake inhibitors (SSRIs), are more specific than the TCAs. SSRIs block the reuptake of serotonin, making more available in the synapse, with little effect on the reuptake of norepinephrine (Figure 18.3).

The SSRIs include fluoxetine (Prozac, human label), paroxetine (Paxil), sertraline (Zoloft), fluvoxamine

(Luvox), citalopram (Celexa), and escitalopram (Lexapro). All are approved by the FDA for treatment of depression in humans. SSRIs are efficacious for the treatment of panic disorder in humans (Tollefson and Rosenbaum, 1998). Fluoxetine was approved by the FDA for treatment of dogs (Reconcile, veterinary brand), but the company withdrew the product from marketing. The FDA approval supported efficacy and safety in dogs (Simpson et al., 2007). Often a low dose is used to initiate therapy, and then it is titrated upward as necessary (Zahajzky et al., 2009). Most SSRIs have been shown to be effective in treatment of human obsessive–compulsive disorder (Tollefson and Rosenbaum, 1998).

In dogs, SSRIs have been used clinically for management of separation anxiety (Simpson et al., 2007), compulsive behaviors, and dominance-type (Dodman et al., 1996a) or impulsive aggression (Peremans et al., 2005). In cats, SSRIs are used to treat urine spraying, aggression, and compulsive behavior such as psychogenic alopecia and fabric chewing. Due to concerns about GIT adverse effects, cats medicated with SSRIs should be closely monitored for food and water consumption, and fecal and urinary elimination, as well as body weight. In horses, there is little known about the metabolism and effects of SSRIs.

There is evidence that individuals of several species who exhibit violent behavior have low CNS serotonin activity, as measured by the CSF concentrations of serotonin metabolites (rhesus macaques, Mehlman et al., 1994; dogs, Reisner et al., 1996). Therefore, serotonin-enhancing agents have been used to treat certain forms of aggression, particularly dominance-related aggression (Dodman et al., 1996a; Peremans et al., 2005).

Nonbehavior modifying effects: As discussed for the TCAs, antidepressants have been used for treatment of pain (Micó et al., 2006). However, the strongest evidence has been for TCA drugs, which have effects on neurotransmitters (norepinephrine, histamine, dopamine) other than serotonin. Although there is some evidence for an analgesic effect from SSRI drugs, it is not as convincing as for TCA drugs.

Adverse effects: In general, SSRIs have an excellent safety record. Adverse effects vary from agent to agent, but include gastrointestinal effects and nervous system alterations ranging from sedation to agitation, irritability, and insomnia. In a study of fluoxetine in dogs (Simpson et al., 2007) in which 122 dogs were treated with fluoxetine and 120 with placebo, the most common adverse effect was lethargy/depression/calming (45%). The second most common effect reported was anorexia/decreased appetite (29%). Gastrointestinal effects (up to 25% of humans) are likely due to the concentration of serotonin receptors in the GIT. SSRIs are considered to cause less effect on cardiac impulse conduction than TCAs (Pacher et al., 1999). Starting a patient at a low dose and then increasing after 1 week may reduce the likelihood of adverse events (Sherman, pers. obs.). Onset to action may be 1–4 weeks (Simpson et al., 2007). Because of its extended half-life, fluoxetine in humans (Zahajzky et al., 2009) and dogs (FDA, 2007) does not require dose tapering for discontinuation.

In people, there is great variation in the effect of SSRIs on CYP 450 enzymes. These differences have not been reported in animals. In people, fluoxetine, fluvoxamine, and paroxetine all inhibit one or more CYP enzymes; sertraline, citalopram, and escitalopram do not. But, as discussed in Section Absorption, Metabolism, Clearance, and Distribution, it is not known what CYP 450 enzymes are affected by these drugs compared to people because of the differences in enzymes and it is difficult to predict potential drug interactions. Drug interactions have been infrequently reported from administration of an SSRI to animals.

Fluoxetine

Fluoxetine (Prozac, and generic human label) is widely used to treat a range of human behavioral disorders, including depression, generalized anxiety, panic disorder, obsessive–compulsive disorder, eating disorders, and premenstrual dysphoria (Figure 18.4).

Among dogs, fluoxetine has been used for treatment of dominance-related aggression (Overall, 1999a; Dodman et al., 1996a), interdog aggression (Dodman, 2000), acral lick dermatitis (Rapoport et al., 1992; Wynchank and Berk, 1998a), anxiety and panic-like disorders, as well as other compulsive disorders (Irimajiri and Luescher, 2005). Fluoxetine has also been used to successfully treat stereotypical pacing (considered a compulsive disorder) of 22 years' duration in a captive polar bear; relapse occurred after discontinuation of treatment (Poulsen et al., 1996). Among cats, fluoxetine has been used for treatment of refractory urine spraying (Pryor et al., 2001), inappropriate urination (Romatowski, 1998), psychogenic alopecia (Hartman, 1995; Romatowski, 1998), and aggression (Overall, 1999b; Romatowski, 1998).

Fluoxetine is metabolized to norfluoxetine, its active metabolite. Norfluoxetine has an elimination half-life of 4–16 days in humans (Zahajzky et al., 2009). After a single dose in dogs, fluoxetine has an elimination half-life of 3–13 hours; norfluoxetine has an elimination half-life of 33–64 hours (Reconcile package insert, Lilly, 2007). This long half-life offers protection from the discontinuation syndromes associated with abrupt interruption or termination of treatment. It also necessitates a washout period after discontinuation of fluoxetine and initiation of a monoamine oxidase inhibitor such as selegiline (Zahajzky et al., 2009; Reconcile package insert, Lilly, 2007).

Because the approved canine form is no longer available, veterinarians use the human generic formulations.

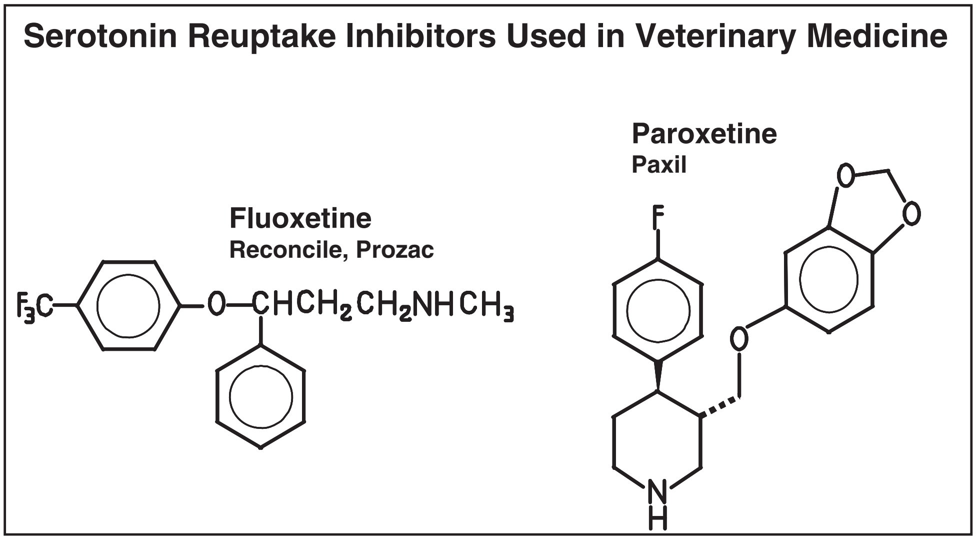

Figure 18.4 Selective serotonin reuptake inhibitors (SSRIs) used in veterinary medicine. Fluoxetine (left) and paroxetine (right). Only fluoxetine (Reconcile) has been licensed for veterinary use.

The 10 and 20-mg capsules must be divided or compounded for smaller animals, or 10 or 20-mg tablets that can be broken. There is a 4 mg/ml oral liquid solution, but it is mint flavored and has 0.23% alcohol, which some animals (especially cats) may find unpalatable. The usual dose for dogs is 1–2 mg/kg oral per day (Simpson et al., 2007); the dose for cats is 0.5–1.0 mg/kg oral per day (Landsberg et al., 2013; Pryor et al., 2001). Fluoxetine may require 1–4 weeks to effect.

In dogs the most common effects in clinical trials were sedation and calming effects, followed by anorexia and weight loss. Less common effects observed were vomiting, shaking, diarrhea, restlessness, vocalization, aggression, and, in rare cases, seizures. Treatment with fluoxetine in dogs for acral lick dermatitis was well tolerated (Wynchank and Berk, 1998b). In dogs and cats, inappetence is a common side effect (Pryor et al., 2001; Simpson et al., 2007); lethargy has been reported (Simpson et al., 2007; Hartman, 1995; Pryor et al., 2001); vomiting is rare (Pryor et al., 2001). Fluoxetine may decrease libido in breeding animals; higher doses of fluoxetine may cause serotonin effects in the intestine.

Paroxetine

Like fluoxetine, paroxetine is used in humans to treat a range of psychiatric complaints, including depression, social anxiety, and panic disorder (Figure 18.4) and has been considered by some to be the first choice for treatment of generalized anxiety (Baldwin and Tiwari, 2015).

In dogs, paroxetine may be helpful for the treatment of canine aggression and canine compulsive disorders but efficacy has not been documented as well as for fluoxetine. In cats, paroxetine has been used for compulsive behaviors, redirected aggression, and generalized anxiety. Paroxetine is available in 10, 20, 30, and 40-mg tablets that can be more easily divided than capsules and administered orally to cats.

Compared to fluoxetine, paroxetine has a shorter elimination half-life and reaches steady-state more rapidly in people. However, pharmacokinetics have not been fully reported for animals.

Compared to fluoxetine, anticholinergic side effects, such as dry mouth and constipation, are more common with paroxetine. Constipation is a common anticholinergic side effect of paroxetine use in cats. Cats should be carefully monitored for food and water consumption and urine and feces production during the first week of therapy. Reducing the target dose by half for the first week can avoid such side effects (Sherman, pers. obs. 2000–2006). Weight gain is a common side effect in cats with paroxetine treatment (Sherman, pers. obs. 2000–2006). To avoid negative metabolic consequences of weight gain, the body weight of cats receiving paroxetine treatment should be monitored closely. Paroxetine can cause an idiosyncratic, dose-dependent increase in arousal, wakening, and REM suppression in humans (Ehmke and Nemeroff, 2009) and dogs (Sherman, pers. obs. 2000–2016).

Other SSRIs

Sertraline is an SSRI with pharmacokinetic properties in humans similar to paroxetine. Like other SSRIs, in humans, it is used for a range of behavioral disorders including panic disorder, chronic depression, compulsive disorders, and anxiety (Sheikh et al., 2000). It is considered by some to be the first choice for treatment of panic disorder in humans (Baldwin and Tiwari, 2015) and may provide some cognitive benefits to elderly patients, compared to other SSRIs. Like other SSRIs, sertraline has been used as a treatment for eating disorders. In one report, sertraline was used to successfully treat chronic regurgitation, deemed to be a compulsive behavior in a chimpanzee (Howell et al., 1997). In dogs, **sertraline** (Block et al., 2009) can be helpful for compulsive behaviors (Rapoport et al., 1992), aggression (Larson and Summers, 2001), and anxiety disorders. Diarrhea is a common side effect, which may be circumvented by starting the drug at a low dose and increasing over 2 weeks.

Fluvoxamine (Aboujaoude and Kalin, 2009) is distinguished by its lack of activity in humans at CYP2D6 isozyme. Therefore in people it has been more safely combined with TCAs. However, the use of fluvoxamine is unreported for animals.

Citalopram (Roseboom and Kalin, 2009) was approved by the US FDA in 1998 for the treatment of depression in humans, after a long history of safe human administration in Europe (Pollock, 2001). Although early studies of citalopram administration to dogs at high doses (20 mg/kg) (Boeck et al., 1982) revealed no cardiac effects, one toxicity study of 10 beagle dogs given a high dose of citalopram (8 mg/kg/day) for 17–31 weeks suffered 50% mortality due to cardiac effects (Forest Pharmaceuticals Inc., 1998). Citalopram has been used to treat acral lick dermatitis in dogs at a dose of 0.5–1.0 mg/kg/day (Stein et al., 1998). Citalopram may modulate aggressive behavior in humans (Reist et al., 2003) and dogs. One study of impulsively aggressive dogs (N = 9) treated for 6 weeks with citalopram demonstrated behavioral improvement (Peremans et al., 2005). Based on the suggestion of increased cardiac sensitivity to citalopram particularly in beagle dogs, cardiac screening (Pacher et al., 1999) and conservative dosing, or alternative drug selection are recommended. To date, there are no published reports of the use of citalopram in cats.

The S-isomer of racemic citalopram, **escitalopram** (Lexapro, Forest Pharmaceuticals), may be promising for the treatment of behavioral disorders in dogs, but at this time, there is limited information. It is a potent and highly selective inhibitor of CNS neuronal reuptake of serotonin (Roseboom and Kalin, 2009). No published studies are currently available for its use in companion animals. It is now available as a generic, but future reports will be needed to determine efficacy in companion animals.

Other and Atypical Antidepressants

Serotonin and norepinephrine reuptake inhibitors (SNRIs) include venlafaxine and duloxetine (Thase and Sloan, 2009), among others. To date, they are uncommonly used in the treatment of behavioral disorders in companion animals (dogs and cats) and research on their effectiveness is lacking. Given their ability to affect both serotonin and norepinephrine reuptake, these have been used to treat pain syndromes, particularly neuropathic pain and fibromyalgia, but application in veterinary medicine has not been well characterized. The atypical antidepressants are a diverse category of heterocyclic drugs. These include trazodone (Desyrel), nefazodone (Serzone), bupropion (Welbutrin, Welbutrin XL, Zyban), and mirtazapine (Remeron). Because of its rare association in humans with life-threatening liver failure, nefazodone is now rarely prescribed. Buproprion has a stimulating effect, and does not sufficiently enhance behavioral calming in animals to be clinically useful. Discussed below are the other atypical antidepressants trazodone and mirtazapine.

Trazodone: Most notable in this group for clinical use in small animals is trazodone (Golden et al., 2009). Trazodone is a mixed serotonergic agonist/antagonist. It has no anticholinergic effects, moderate antihistaminergic activity, and is an antagonist of postsynaptic α_1-adrenergic receptors. Trazodone is well absorbed in humans and appears to have similarly rapid and complete oral absorption in animals. Trazodone is bioavailable after oral administration in dogs, though variation exists in time to maximum plasma concentrations (Jay, 2013). Pharmacokinetic data is not available in cats, but behavioral effects may be seen fairly rapidly following oral administration in cats (Orlando, 2016), and plasma concentrations were achieved that were in the range produced in dogs and humans.

In humans, the cytochrome isozyme CYP2D6 is involved in the metabolism of trazodone and caution is advised when prescribing trazodone with SSRIs that inhibit CYP2D6 (Golden et al., 1998). However, the wide safe dose range for trazodone makes this less problematic than other drugs, such as the TCAs. Trazodone is used in humans for treatment of major depression and to counter the sleep disturbances caused by SSRIs. When given over weeks, it produces anxiolytic properties similar to diazepam (Golden et al., 1998). An extended-release formulation of trazodone (Oleptro) is now available for treatment of depression in humans, but has not been evaluated in animals.

Trazodone has been used in dogs for mild thunderstorm phobia (Gruen and Sherman, 2012) and as an adjunct to TCA or SSRI treatment (Gruen and Sherman, 2008). However, trazodone may have some limitations. In one study of four “depressed” laboratory beagles, no effect was noted after administration of trazodone at a high dose (10 mg/kg; Iorio et al., 1983); its effects appear insufficient in severe cases of thunderstorm phobia (Sherman, pers. obs. 1997–2002). Side effects in dogs include sedation and GIT issues, including vomiting and diarrhea (Sherman, pers. obs. 1997–2002), particularly during the first few days of dosing. Starting at a low dose and titrating the dose up over the initial days and subsequent weeks may be helpful (Gruen and Sherman, 2008). Priapism, noted as a rare side effect in human males, has not been observed in neutered male dogs (Sherman, pers. obs. 1997–2007).

Trazodone may be used in combination with antidepressants, particularly SSRIs, to enhance behavioral calming, decrease agitation, and to aid with sleep. This can be helpful in humans with depression, obsessive–compulsive disorder, and other anxiety disorders (Stahl, 2013). It may be similarly effective in dogs (Gruen and Sherman, 2008). Trazodone has also been shown to be effective in facilitating behavioral calming in dogs following elective orthopedic surgeries (Gruen et al., 2014) and in cats for travel anxiety and veterinary examinations (Stevens et al., 2016).

Mirtazapine: Mirtazapine (Remeron) exhibits noradrenergic and serotonergic activity through its activity as an α_2 antagonist. In humans, it is used to manage depression and anxiety. Mirtazapine is a potent antagonist of histamine H_1 receptors, which may account for its sedative effects. The antagonism of $5HT_3$ receptors by mirtazapine results in an antiemetic effect, suggesting that mirtazapine could be considered a successful antiemetic agent in patients suffering from nausea and vomiting. Although uncommonly used for the treatment of primary behavior problems in animals, it has recently found safe and useful application in the management of anorexia in dogs and cats. Elimination half-life in dogs ranges from 20 to 40 hours and reaches a steady-state in 4–6 days.

The most common use of mirtazapine has been for appetite stimulation in cats, particularly cats with chronic kidney disease (CKD). The appetite-stimulating effects are attributed to the $5HT_3$ antagonist properties; therefore, it may share similar effects as antiserotonin medications used for antiemetic uses. Mirtazapine has been studied more in cats than in dogs (Quimby and Lunn, 2013). These studies in cats have shown that it is an effective appetite stimulant. The dose is usually 1.88 mg per cat oral. At high doses it produces adverse effects that include vocalization and increased restlessness. In cats it has a half-life of approximately 10 hours, which allows for once-daily dosing. In cats with chronic kidney disease – for which appetite stimulation often is desired – clearance is slower and the half-life increases to 15 hours, which indicates that an every-other-day dosing schedule of 1.88 mg per cat should be used in cats with kidney disease to avoid accumulation (Quimby et al., 2011). Mirtazapine is available as 7.5-mg tablets, but also is available in 15, 30, and 45-mg sizes. The formulation that some veterinarians prefer is a rapidly disintegrating oral tablet that dissolves easily in an animal's mouth (15, 30, and 45 mg).

Monoamine Oxidase Inhibitors

Monoamine oxidase inhibitors (MAOIs) are drugs that inhibit the intracellular enzyme monoamine oxidase (MAO) (Krishnan, 2009). Since MAO catabolizes intracellular monoamine neurotransmitters, such as serotonin, dopamine, norepinephrine, and tyramine, MAOIs inhibit this process, causing an increase in monoamines. There are two MAO subtypes, A, which affects serotonin, dopamine, norepinephrine, and tyramine, and B, which affects the metabolism of phenylethylamine as well as dopamine. Location of the enzymes also characterizes the differences between type A and B. Type A is located in the intestine as well as the CNS. If MAO-type A is inhibited, it can produce a decrease in the metabolism of compounds from foods that can produce systemic effects. However, this effect has not been well characterized in animals, perhaps because of the rare clinical use of these drugs.

Selegiline

The nonselective (Type A and B) MAO-inhibitors are not used to any extent in veterinary medicine. Only one MAO-B inhibitor, selegiline (L-deprenyl, Anipryl), is clinically important as a behavioral treatment in animals. Selegiline is approved by the FDA for treatment of canine cognitive dysfunction, a disorder of elderly dogs characterized by decreased social interactions, loss of house-training, confusion, and changes in sleep cycle (Milgram et al., 1993; Ruehl et al., 1995). There is evidence that these behavioral changes have a histolopathological (Cummings et al., 1996) and metabolic (Milgram et al., 1993) basis and selegiline may affect these changes. However, at currently registered doses for dogs, it has not produced beneficial effects on anxiety or depression and is generally not used for this purpose by veterinary behavior experts in the United States.

The action of selegiline is to inhibit MAO type B (and other MAOs at higher doses). Studies in dogs showed that MAO inhibition was specific at doses of 1 mg/kg (Milgram et al., 1993, 1995). The proposed mechanism of action of this drug is to inhibit the metabolism of dopamine in the central nervous system (Bruyette et al., 1995). The theory to explain the beneficial response is that dopamine depletion in the brain and loss of dopaminergic neurons leads to cognitive dysfunction (dementia) in old dogs. Treatment with selegiline increases dopamine concentrations in the brain, which restores neurotransmitter balance and improves cognitive ability. It also inhibits the metabolism of phenylethylamine (Milgram et al., 1993, 1995). Phenylethylamine in laboratory animals produces amphetamine-like effects. There are two active metabolites of selegiline, L-amphetamine and L-methamphetamine. In dogs, even though there were increases in amphetamine concentrations (Milgram et al., 1993), they were not high enough to produce adverse effects. However, at high doses (>3 mg/kg) the authors proposed that increased amphetamine concentrations could explain the observed behavioral changes. The L-isomer metabolites are not as active as their D-forms and studies have not supported a potential for amphetamine-like abuse or dependency from selegiline, compared to other amphetamine-like drugs.

In clinical trials, selegiline significantly improved clinical signs of cognitive dysfunction in treated dogs compared to controls. Selegiline may also improve learning (Mills and Ledger, 2001). In general, selegiline is given daily for a month. Any improvement in clinical signs dictates continuation of treatment; additional improvement is often seen in subsequent months (Calves, 2000). Treatment failure should prompt an increase in daily dose and an additional trial of medication. As an extralabel application, selegiline has also been used in geriatric cats diagnosed with cognitive dysfunction.

Side effects are uncommon, but high doses can cause hyperactivity and stereotypic behavior in dogs. In studies in dogs in which selegiline was administered a doses of 1, 4, or 16 mg/kg for 1 year, adverse effects were rare. At high doses salivation, panting, repetitive movements, decreased weight, and changes in activity level were noted. In one report (Milgram et al., 1993), changes in behavior were not seen until doses exceeded 3 mg/kg, which the authors attributed to amphetamine-like effects.

Selegiline is also FDA-approved for treating canine pituitary-dependent hyperadrenocorticism (PDH). The mechanism of action to explain selegiline's effect for treating PDH is potentiation of dopamine. Increased dopamine levels in the brain decreases ACTH release, resulting in lower cortisol levels (Bruyette et al., 1995, 1996). Blockade of dopamine enhances CRH-mediated ACTH release in normal dogs. In 52 dogs with PDH, administered selegiline at 1.0 mg/kg once daily, there was significant improvement in polyuria–polydipsia, appetite, activity, thinning of skin and alopecia, body weight, and body conformation (Bruyette et al., 1996). There was also a significantly lower mean response in the low-dose dexamethasone suppression test. Overall, selegiline was evaluated as effective in 71% of dogs and not effective in 21%. Side effects of therapy were rare. However, other studies released after the drug's FDA approval have not been as convincing. In one study, the authors found a much smaller percentage of PDH dogs that responded to selegiline and they did not recommend this drug for treatment (Reusch et al., 1999). It has been proposed that only dogs with *pars intermedia* lesions of the pituitary will respond to selegiline, which represents 30% or less of canine PDH cases (Peterson, 1999).

MAOIs can sufficiently inhibit catabolism of monoamines that their concentration, particularly serotonin, becomes toxic. This may occur when other antidepressants that inhibit reuptake of serotonin or inhibit MAO are used concurrently. This can lead to serotonin syndrome, a potentially fatal condition, characterized by hypertension, hyperthermia, restlessness, tremor, seizures, and altered mental status (Calves, 2000; Krishnan, 2009; Wismer, 2000). Thus, an MAOI should not be used with an antidepressant, including TCAs (Anderson, 2001) and SSRIs. Drugs from one class should be discontinued 14 days before agents from the other class are initiated (Anderson, 2001). Tricyclic antidepressants and selective serotonin reuptake inhibitors should not be used concurrently with other MAOI such as amitraz, a topical product used for control of ticks and mites.

Because selegiline may affect other monoamine synthesis, there has been concern about its administration with sympathetic amines used to treat urinary incontinence in dogs, such as phenylpropanolamine. However, when this was studied, selegiline had no effect on pulse rate, ECG, or behavior compared to administration of phenylpropanolamine alone in dogs (Cohn et al., 2002).

Concurrent use of α_2 agonists, phenothiazines, and opiate analgesics is discouraged with selegiline. A standard recommendation is to wait at least five times the elimination half-life of the SSRI or its active metabolite (whichever is longer), before administering the next serotonergic agent (Tollefson and Rosenbaum, 1998). A washout period of 1–3 weeks is recommended after discontinuation of an MAOI and initiation of another drug that affects monoamines.

Miscellaneous Drugs

Anticonvulsant Drugs

Anticonvulsants are covered in more detail in Chapter 17. However, they are mentioned briefly here because some anticonvulsants have been used to manage veterinary behavior problems. Some maladaptive behaviors, such as tail chasing and unprovoked rage aggression in Bull Terriers, have been postulated to be partial complex seizures at least in part due to their positive response to phenobarbital (Crowell-Davis et al., 1989; Dodman et al., 1992, 1996b). In other cases with similar presentations, phenobarbital treatment is ineffective (Brown et al., 1987). Response may be obtained with narcotic antagonists (Brown et al., 1987) or anticompulsive agents (Hewson, 1998b).

Other applications exist for the behavioral use of anticonvulsants. Carbamazepine (Tegretol) has been used for the treatment of aggression in two cats (Schwartz, 1994) and for treatment of aggression (Carter, 2008) and psychomotor seizures in a dog (Holland, 1988). Since blood dyscrasias may result (at least in humans), regular evaluation of the CBC is recommended for patients given carbamazepine. New anticonvulsants, such as gabapentin (Neurontin), topiramate (Topamax), lamotrigine (Lamictal), and tiagabine (Gabitril), show promise for management of anxiety disorders and other behavioral disturbances. Gabapentin, in particular, is used for treatment of anxiety in both dogs and cats, and may be particularly useful when concerns exist about underlying pain.

Opiates

Opiates are presented in more detail in Chapter 13. Stereotypic, compulsive behaviors, such as self-traumatic licking, tail chasing, and pacing, are seen in animals confined to zoos and laboratory settings (Kenny, 1994). It has been postulated that such behaviors are "coping strategies" that lead to release of endogenous opiates (endorphins). Some cases of dogs and other animals have been successfully treated with the oral narcotic antagonist naltrexone (Brown et al., 1987; Dodman et al., 1988; Kenny, 1994; White, 1990) or other opiate antagonists (Dodman et al., 1988). Horses with "crib biting" have also been treated with opiate antagonists.

There is evidence that there may be a developmental component to the expression of stereotypies (Kennes et al., 1988). Other cases of client-owned dogs develop acral lick dermatitis associated with repetitive licking for unknown reasons (Dodman et al., 1988; White, 1990). Since the etiology of stereotypic behavior (based on response to treatment) is uncertain, treatment with anticompulsive (Hewson et al., 1998b) or anticonvulsant (Dodman et al., 1992) agents should also be considered in such cases.

Hormonal Therapy

Reproductive hormones are discussed in more detail in Chapter 27. Historically, synthetic progestins have been used to treat a wide range of behavioral disorders. Most likely the response to these drugs is caused by their mild sedating effects on steroid receptors in the CNS (McEwen et al., 1979). This nonspecific use has declined with the advent of more specific agents, described in this chapter. The use of progestin hormones for behavioral therapy is now considered a "last resort" therapy to avoid abandonment or euthanasia of the offending animal.

In castrated and entire male dogs, megestrol acetate (Ovaban) has been used for the treatment of dominance-type (Borchelt and Voith, 1986) and intermale aggression, mounting, urine marking, and tendency to roam (Joby et al., 1984). In one study of 123 males, 75% improved with hormone treatment (Joby et al., 1984). Side effects included increased appetite and lethargy. Relapse within 3 months was most common in dogs that had signs of dominance-type aggression and marking (Joby et al., 1984). However, such treatment significantly impairs adrenocortical function as measured by ACTH stimulation during treatment (van den Broek and O'Farrell, 1994).

In cats, megestrol acetate may reduce the incidence of aggression (Henick et al., 1985; Marder, 1993) and urine spraying, particularly among neutered male cats (Hart, 1980). Side effects of progestins in cats include hyperphagia, obesity, hyperglycemia leading to diabetes, lethargy, mammary gland hyperplasia and adenocarcinoma, pyometra, and bone marrow suppression (Romatowski, 1989; Henik et al., 1985).

Another class of drugs that has been used in male animals for treatment of behavior problems are long-acting progestins. Medroxyprogesterone acetate (DepoProvera) is the most common of this group. It has a long-acting depot effect after a single injection that may last several days up to 3–4 weeks. A dose that has been used is 10–20 mg/kg, injected SC or IM. This drug may be as effective as megestrol acetate for some indications. Medroxyprogesterone acetate (Depo-Provera) has been used in cats for urine spraying in a manner similar to that of megestrol acetate (Hart, 1980).

Reproductive hormones have been used in postmenopausal women in combination with a first-line SSRI to enhance therapeutic effects (Stahl, 2013). Although controlled clinical trials are not available, clinicians anecdotally report benefits. Such a strategy may be helpful in spayed female dogs with refractory behavioral problems, although no published reports are currently available.

Thyroid hormones are discussed in Chapter 28. Thyroid hormones have been used to accelerate (Altshuler et al., 2001) or augment (Stahl, 2013) TCA response, particularly in women. Thyroid supplementation may increase cortical 5HT concentrations and desensitize autoinhibitory $5HT_{1A}$ receptors in the raphe area, resulting in disinhibition of cortical and hippocampal 5HT release (Altshuler et al., 2001). Mixed reports exist about the association between thyroid hormone concentrations and the effect of thyroid supplementation in dogs with aggression.

Melatonin is produced by the pineal gland according to the light/dark cycle, with light acting as a production suppressant. The nocturnal secretion of melatonin is primarily induced by increased noradrenergic neurotransmission, resulting in increased activity of the rate-limiting enzyme that converts serotonin to melatonin. In recent years, melatonin has become a popular over-the-counter remedy for insomnia in humans and behavioral calming in dogs, although data on safely, efficacy, or appropriate doses are not available (Stahl, 2013). Melatonin given with amitriptyline was used in one dog to improve signs of generalized anxiety disorder (Aronson, 1999).

Melatonin is an endogenous hormone, produced from serotonin in the pineal gland. At high doses in dogs (1–1.3 mg/kg q 12 h), melatonin affects endogenous sex hormones, but does not affect prolactin or thyroid concentrations in adult dogs (Ashley et al., 1999). Melatonin has been used with amitriptyline to manage thunderstorm phobia (Aronson, 1999), since it impairs psychomotor vigilance (Graw et al., 2001). No controlled studies on the behavioral effects of melatonin on dogs have been conducted.

Beta-Blockers

The beta-adrenergic antagonist propranolol (Inderal) has been used to ameliorate the sympathetic symptoms of anxiety in humans, including trembling, sweaty palms, and tachycardia (reviewed in Steenen et al., 2016) and to treat organically based aggression (Williams et al., 1982). No controlled studies have investigated the efficacy of beta-blockers for treatment of anxiety disorders in small animals (Shull-Selcer and Stagg, 1991).

Pindolol is a beta-adrenergic blocker that is also an antagonist and partial agonist at $5HT_{1A}$ receptors. Pindolol may disinhibit serotonin neurons and may serve as a useful adjuvant therapy (Stahl, 2013).

In Great Britain, propranolol is used in combination with phenobarbital to manage phobic behavior (Walker et al., 1997). When propranolol is used in combination with the antipsychotic thioridazine, thioridazine plasma levels increase.

A beta-blocker with $5HT_{1A}$ autoreceptor antagonist properties, pindolol, may disinhibit serotonin neurons and may serve as a useful adjuvant therapy to TCAs (Altshuler et al., 2001; Stahl, 2013). However, in one study, pindolol was not superior to placebo for augmenting the effects of paroxetine of social anxiety symptoms (Stein et al., 2001).

Alpha$_2$ Agonists

These drugs are discussed in more detail in Chapter 14. The α_2-adrenergic agonists may be used for pharmacological behavioral management. Detomidine and dexmedetomidine, widely used for sedation via parenteral routes in animals, have significant behavioral effects when used via oral transmucosal (OTM) administration. Because of high first-pass metabolism effects these agents are not be active when swallowed.

Detomidine gel is marketed for OTM use in horses (Dormosedan Gel, Zoetis), particularly as a sedative and analgesic for standing procedures (l'Ami et al., 2013). When given in the buccal pouch of laboratory dogs, the equine formulation of detomidine (0.35 mg/m^2 OTM) has been shown to produce sedation, reduce anxiety, and facilitate handling in laboratory dogs (Hopfensperger et al., 2013). Side effects included transient bradycardia but no respiratory depression.

Dexmedetomidine has been given via OTM route to dogs (Cohen and Bennett, 2015) and cats (Santos et al., 2010) for procedural sedation. The injectable formulation of dexmedetomidine (Dexdormetor, Zoetis) was administered in the buccal pouch to dogs (N = 4, mean dose 32.6 μg/kg body weight), resulting in satisfactory sedation for restraint, and subsequently reversed with atipamezole. OTM dexmedetomidine (20 μg/kg) plus buprenorphine (20 μg/kg) were used to sedate 87 shelter-owned cats for catheter placement (Santos et al., 2010). In this study, 25% of the cats required additional use of IM ketamine to facilitate catheterization.

Recently, dexmedetomidine gel has been formulated for OTM administration in dogs and is now approved by the FDA to attenuate fear and/or anxiety elicited by specific noises, such as the sound of fireworks (Sileo, Zoetis). The recommended dose, given 30–60 minutes prior to the inciting noise, is 125 μg/m^2 OTM, with redosing after 2 or more hours. In a double-blinded, placebo controlled clinical trial (N = 144 dogs), 75% of owners whose dogs received Sileo rated the treatment effect to be good or excellent (FOI information, Sileo). Published data for the Sileo oral gel product is not available for cats. However, oral transmucosal administration of dexmedetomidine has been used in cats at a dose of 40 μg/kg and was safe and effective. It produced similar sedative and antinociceptive properties as the parenteral administration (Slingsby et al., 2009).

The centrally acting α agonist clonidine is used in human medicine as an antihypertensive agent, and to target behavioral signs associated with hyperactivity, hypervigilance, impulsivity, and posttraumatic stress syndrome (Strawn and Geracioti, 2008). Clonidine blocks norepinephrine release through activation of α_2 receptors on presynaptic neurons in the locus ceruleus. In dogs, clonidine has been used orally to manage fear-based problems in dogs, often in combination with other serotonergic agents. In one open trial of 22 client-owned dogs, 82% of clients reported positive effect with the addition of clonidine (Ogata and Dodman, 2011). The dose range was 0.007–0.049 mg/kg PRN up to twice a day PO; some patients also received clomipramine, fluoxetine, sertraline, or buspirone (Ogata and Dodman, 2011).

Tramadol

Tramadol is discussed more in Chapter 13. Tramadol has three effects that may act synergistically: opiate agonist, inhibition of synaptic reuptake of norepinephrine, and inhibition of synaptic reuptake of serotonin. The latter two effects resemble the action of tricyclic antidepressants discussed earlier in this chapter. With respect to efficacy of tramadol for some conditions – including behavior problems – perhaps equal or greater importance than opiate agonistic activity is the effect on serotonin and norepinephrine reuptake. One of the stereoisomers is more prominent at inhibiting serotonin reuptake and the other isomer has more of an effect on norepinephrine reuptake. Some studies in experimental animals and people with tramadol have elicited significant behavior-modifying effects. However, veterinary clinical studies for this use are lacking.

Analgesia may also play a role in the action of this drug for some behavior problems. Veterinary behaviorists are often faced with uncertainty about the role of pain in the presentation of a behavior problem. Older animals or those with chronic degenerative orthopedic diseases may be irritable, leading to anxiety or irritable aggression. Although a thorough physical examination and appropriate diagnostic tests are indicated, the role of pain may remain uncertain. Often, a trial of analgesics may be initiated as a way to determine if pain management improves behavior. In addition to the many new nonsteroidal agents discussed in Chapter 20, tramadol also may be useful, alone or in combination with other behavioral drugs.

Nutraceuticals

A range of products often called nutraceuticals, natural products, or dietary supplements are marketed for treatment of behavior disorders in dogs and cats and are available to pet owners without a veterinarian's prescription. These products may be used alone or in combination with pharmaceutical therapy. The most common applications in dogs and cats are for management of fear and anxiety, and for management of cognitive dysfunction. Briefly summarized here are commercially available products with efficacy supported by one or more studies in companion animals.

Natural products used to treat fear and anxiety include amino acids, plant proteins, and milk proteins. L-theanine, is an analogue of the amino acid L-glutamate. It is considered safe by the FDA and is found primarily in particular plant and fungal species, including green tea. A veterinary formulation (Anxitane, Virbac Animal Health) has been shown to reduce fear of humans in laboratory dogs (Araujo et al., 2010) and to reduce fear responses to thunderstorms in client-owned dogs (Pike et al., 2015). Another product, combining extracts from plants *Magnolia officinalis* and *Phellodendron amurense* (Harmonese, Vet Products Labs) has been shown to reduce anxiety associated with thunderstorm sounds in laboratory dogs (DePorter et al., 2012). α-casozepine, a lactose-free milk protein (Zylkene, Vetoquinol) has been shown to reduce signs of anxiety-related disorders in cats (Beata et al., 2007a) and dogs (Beata et al., 2007b). In one blinded study, there was no difference in efficacy between α-casozepine and the monoamine oxidase inhibitor selegiline (Anipryl) for treatment of anxiety disorders in dogs (Beata et al., 2007b).

Combinations of these products are commercially available. One product combines L-theanine, colostrum complex, and thiamine (B1) (Composure, VetriScience); another combines L-theanine, *Magnolia officinalis*, *Phellodendron amurense*, and whey milk protein (Solliquin, Nutramax Labs). Clinical studies of these products are not available; anecdotal reports suggest that they are well tolerated by dogs and cats (Sherman, personal communication, 2016).

Natural products are marketed for management of age-related cognitive dysfunction. These include *S*-adenosylmethionine (SAMe) (Novifit, Virbac Animal Health). SAMe is an endogenous metabolite involved in several biochemical pathways, and is deficient in humans with Alzheimer's disease. In a laboratory study of aged dogs and cats treated with SAMe, dogs showed measurable reduction in learning errors but no treatment effects on memory (Araujo et al., 2012). Cats with relatively low levels of cognitive impairment showed a reduction in learning errors with treatment but cats with high levels of impairment showed no such beneficial effects. The results suggested treatment early in the disease course may be most effective (Araujo et al., 2012).

Apoaequorin, a calcium-buffering protein obtained from jellyfish (Neutricks, Neutricks LLC), enhanced operant learning in aged dogs with signs of cognitive dysfunction (Milgram et al., 2015). Another product that combines agents *Ginkgo biloba*, phosphatidylserine, D-α tocopherol, and pyridoxine, suggested to have neuroprotective properties on age-related brain neurodegenerative changes (Senilife, Ceva Animal Health). In one study, administration of Senilife to geriatric dogs reduced the frequency of behaviors associated with canine cognitive dysfunction (Osella et al., 2007).

Drug Combinations

Combined Use of Behavioral Drugs

When the effect of one drug is inadequate, a second drug may be added. In fact, this strategy is utilized in commercially available combination products, such as the combination of olanzapine (an antipsychotic) and fluoxetine (an SSRI) marketed by Lilly under the trade name Symbyax, approved by the FDA for treatment of bipolar depression in humans (Eli Lilly, 2017). One strategy is to start with an antidepressant, such as a TCA or SSRI. Then, if needed, a benzodiazepine (Ibanez and Anzola, 2009), trazodone (Gruen and Sheman, 2008), or other agent may be added. A cautious approach is to add the second agent at a low dose, and then titrate the dose to a therapeutic level as response is monitored. Failure to sufficiently manage severe behavior problems, such as separation anxiety, can lead to animal relinquishment and even euthanasia, so aggressive treatment, in combination with behavior modification, may be necessary.

Treatment Success

In each case to which psychotropic medication is administered, there must be a means of documenting treatment response. The first step is to determine target signs that can be documented by the client with regard to frequency, intensity, and duration. The second step is to document the occurrence of these signs over time. Treatment response is often defined as 50% or greater improvement in symptoms.

Clients must also be educated as to probable side effects and duration to effect. Many behavioral drugs will produce side effects within hours or days of first administration, but may require weeks to onset of desired behavioral effects.

The duration of treatment has not been systematically investigated. One strategy is to continue treatment for 2 months after satisfactory treatment response, and then gradually decrease the dose over weeks. If the status quo is maintained, the drug can be discontinued. If treatment success wanes, the previous dose should be reinstated for an additional 6 months and the process repeated.

With the exception of clomipramine (Clomicalm), fluoxetine (Reconcile), and selegiline (Anipryl), the behavioral drugs discussed here are not approved by the FDA for animal use. The limitations and risks of extralabel prescribing should be explained to the pet owner (Simpson and Voith, 1997; Papich and Davidson, 1995). An evaluation of the medical and behavioral history, a physical and neurological examination, and appropriate laboratory tests should precede prescribing any psychotropic agent, and should be repeated at reasonable intervals during treatment. The risk associated with treating animals aggressive to people, especially children, should be carefully considered (Baumgardner, 1997). Patients should be monitored at regular intervals.

Summary

Behavioral pharmacology plays an increasingly important role in veterinary medicine. Drugs traditionally categorized as anxiolytics, antidepressants, anticonvulsants, and hormones may be used to help manage a range of animal behavior problems. Nutraceuticals offer an opportunity for first-line or adjunctive use, and are worthy of more research to determine efficacy in dogs and cats. Knowledge of how these agents act in the body and interact with other agents is imperative for safe, efficacious use.

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Section 4

Autacoids and Antiinflammatory Drugs

19

Histamine, Serotonin, and their Antagonists

Wolfgang Bäumer

The author thanks H.R. Adams for the original chapter upon which this is based.

Histamine

Research on the effects of histamine (2-[4-imidazole]-ethylamine) began more than 100 years ago (Dale and Laidlaw, 1910) and is still not losing its attraction. Rather, histamine has been described as one of the most extensively researched molecules in the field of medicine (Akdis and Simmons, 2006). The first descriptions of histamine actions were contraction of smooth muscle and lowering of blood pressure. In these early publications, histamine was extracted from ergot extract contaminated with histamine-synthesizing bacteria. At the end of the 1920s, histamine was isolated from mammalian tissue and a direct connection to allergic-inflammatory reactions was discovered. Further characterization of histamine action was made possible by the discovery of specific histamine receptor antagonists. The early antagonists were mainly H_1 receptor blockers but it became obvious that these antagonists could not block all histamine actions. This led to the discovery of further histamine receptors (H_2 and H_3 receptors) and in 2000 the latest of the histamine receptors, the H_4 receptor, was cloned and characterized, breaking new ground in histamine research (Skidgel et al., 2011).

Mainly through H_1 receptor (and partly through the H_4 receptor), histamine is involved in anaphylaxis, inflammations and allergies, and certain types of adverse drug reactions. Using the second type of receptor (H_2), it regulates gastric secretion (Morris, 1992). The H_3 receptors modulate neurotransmitter release from neurons (Sander et al., 2008), whereas H_4 receptors participate in inflammation involving modulation of chemotaxis and cytokine secretion of eosinophils, T cells, and antigen-presenting cells into the site of inflammation (Zampeli and Tiligada, 2009). Both, the histamine H_1 and H_4 receptors are involved in the mediation of pruritus (itch) induced by histamine (Rossbach et al., 2011; Kollmeier et al., 2014). Histamine itself is not used therapeutically, but histamine receptor blocking agents (acting as histamine receptor inverse agonists) are commonly used to inhibit effects of endogenous histamine (MacGlashan, 2003).

Sources, Synthesis, and Metabolism of Histamine

Histamine is formed by the decarboxylation of the amino acid L-histidine by the enzyme histidine decarboxylase. Thus, this enzyme is present in all cell types that contain or synthesize histamine.

Histamine is widely distributed throughout mammalian tissue, but concentrations vary considerably in different species; for example quantities of circulating histamine are relatively high in the goat and rabbit but low in the horse, dog, cat, and human. Most of the histamine stored within the body is derived locally from enzymatic decarboxylation of L-histidine. Two general stores of histamine can be identified in mammalian species: the mast cell pool made up of mast cells in tissue and basophils in the blood. The non–mast-cell pool is localized in the gastrointestinal (GI) tract, central nervous system (CNS), skin, and other organs. These two pools differ not only in cellular composition but also in responsiveness to physiological and pharmacological stimuli.

The mast cell pool of highly concentrated histamine is distributed in connective tissue throughout the body. Circulating basophils, free counterparts of fixed-tissue mast cells, also contain high concentrations of histamine and are grouped with mast cells because of basic similarities. Within these two cell types, histamine is synthesized rather slowly and stored tenaciously in secretory granules; hence, the turnover rate is low. Because of the slow turnover rate, mast cell stores are replenished slowly after exposure to a histamine-releasing agent. The mast cell pool represents the histamine that participates in inflammatory responses, allergic phenomena, shock, some adverse drug reactions, and other forms of cellular insult.

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The precise cellular localizations and physiological functions of the non–mast-cell pool of histamine within the gastric mucosa, brain, and skin are still being identified. Histamine in the stomach mucosa – the source of stimulation of acid secretion on H_2 receptors of the gastric parietal cells – is derived from the enterochromaffin-like cells. Histamine in these regions, in contrast to the mast cell pool, undergoes a rapid turnover rate; it is synthesized and released continuously rather than being stored. Portions of this newly synthesized or nascent histamine are present within neural elements, and histamine acts as a neurotransmitter in the CNS. In the gastric mucosa, a "local hormone" action of histamine controls acid secretion.

Histamine's pharmacological actions are brief because of rapid metabolism and distribution into tissues. Biotransformation of histamine involves methylation and oxidation, as shown in Figure 19.1. For most tissues and species the more important is ring methylation to form *N*-methylhistamine catalyzed by the enzyme histamine-*N*-methyltransferase. Most of this metabolite is oxidized to methylimidazole acetic acid by the enzyme monoamine oxidase B. The second pathway is oxidative deamination catalyzed by the enzyme diamine oxidase (histaminase) to form imidazoleacetic acid, which is conjugated with ribose as riboside. Only a small percentage of the primary amine can be acetylated in the GI tract, absorbed, and excreted in urine. Trace amounts of free histamine is also excreted in urine.

Histamine Release

Histamine is highly concentrated in mast cell granules, where it is stored with a heparin–protein complex, proteolytic enzymes, and other autacoids. Release of histamine basically is a two-step process: sudden exocytotic extrusion of granules from the cell and release of histamine from the granules into the interstitial milieu. The latter occurs as an ionic exchange reaction between extracellular cations and molecules of granular histamine. Release can be initiated by a variety of stressful stimuli, including anaphylaxis-allergy, different drugs and chemicals, and physical injury.

Anaphylaxis and Allergy

Hypersensitivity phenomena associated with antigen–antibody reactions evoke active release of histamine from the mast cell pool (type I or immediate hypersensitivity). Free histamine then plays an important role in mediating physiological manifestations of such reactions as vasodilation, itching, smooth muscle contraction, and edema. Other autacoids also participate in tissue responses to hypersensitivity reactions. Signs of histamine involvement in systemic anaphylaxis vary in different species. In carnivores, histamine and anaphylaxis produce pronounced hypotension and hepatomegaly. In rabbits, pulmonary arterioles constrict and the right heart dilates in response to either histamine injection or exposure of a sensitized individual to the appropriate antigen. In

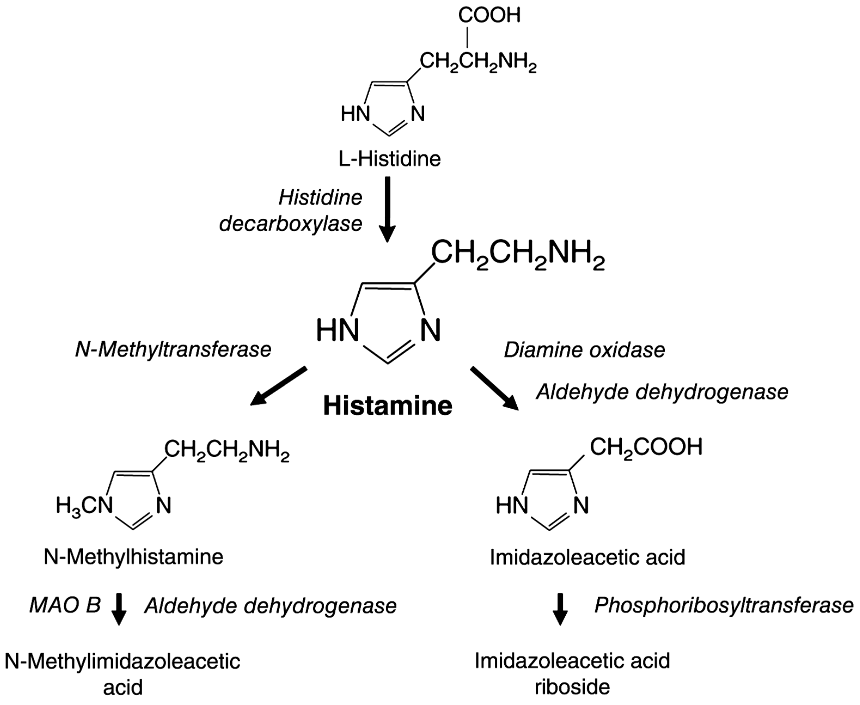

Figure 19.1 Synthesis and metabolism of histamine. Histamine is synthesized from L-histidine by decarboxylation. There are two major metabolizing pathways for histamine. The *N*-methylation of the ring followed by oxidative deamination and the oxidative deamination followed by conjugation with ribose. There seems to be tissue and species specific differences in which pathway is more pivotal; generally the *N*-methylation occurs more often. MAO, monoamine oxidase.

guinea pigs, dominant manifestations are bronchial constriction and death by asphyxiation. Humans seem to respond like guinea pigs and dogs in that severe hypotension, bronchial constriction, and laryngeal edema are principal signs of anaphylaxis.

The mast cell pool of histamine represents a major target for acute types of hypersensitivity-allergy reactions. As part of the allergic reaction to an antigen, IgE antibodies are generated which bind to the high affinity IgE receptor (FCεR1) on mast cells. Upon reexposure of the antigen, a crosslinking of two or more IgE molecules induces a signaling cascade involving tyrosine kinases and phosphorylation of several protein substrates within seconds leading eventually to a mobilization of intracellular calcium, which triggers the exocytosis of contents of secretory granules. Recent findings implicate, that the degranulation and secretion of proinflammatory mediators (cytokines/chemokines and leukotrienes) can be regulated by the affinity of the antigen to the specific IgE antibodies bound to the FCεR1 (Suzuki et al., 2014).

Release is an active process, requiring metabolic energy as well as Ca^{2+}, and should be distinguished from simple release secondary to cell destruction and cytolysis.

The ubiquitous cyclic adenosine 3′,5′-monophosphate (cAMP) system is involved in histamine release evoked by antigen–antibody interactions. An increase in cAMP concentration suppresses histamine release (Lichtenstein and Margolis, 1968). Agents that activate adenylyl cyclase (e.g., catecholamines), inhibit phosphodiesterase (e.g., methylxanthines), or activate β_2-adrenergic receptors on mast cells can be anticipated to inhibit the release of histamine. The beneficial effects of drugs widely used in treating allergic disorders, such as the catecholamines, β_2-agonists, and theophylline, may therefore involve inhibition of histamine release in addition to their well-known and more important physiological antagonism of histamine actions on target cells.

Drugs and Chemicals

Many drugs and chemicals produce direct degranulation of mast cells with release of histamine independently from development of allergy (anaphylactoid reaction). This characteristic action represents an untoward side effect associated either with intravenous (IV) administration of a relatively large dose or direct intradermal injection. Certain chemicals have as their dominant property the ability to release histamine from the mast cell pool.

The curare alkaloids are used clinically as neuromuscular blocking agents (Chapter 10), but they also are notorious for releasing histamine as an adverse side effect; in some species, IV injection of these agents can be followed by histamine-induced bronchospasm and hypotension. Other clinically used drugs that may release histamine include morphine (Guedes et al., 2007), codeine, doxorubicin, vancomycin, and polypeptide antibiotics (polymyxin). However, other opiates such as oxymorphone and hydromorphone are not associated with as much histamine release (Guedes et al., 2006, 2007).

Certain chemicals have been classified simply as histamine-releasing agents because this particular activity supersedes their other pharmacological properties. The best known and most active is the polybasic substance called compound 48/80, a condensation product of *p*-methoxyphenylethylmethylamine with formaldehyde (Goth and Johnson, 1975). Injection of compound 48/80 or other similar agents evokes classic pharmacological signs of histamine release that are susceptible to blockade by antihistaminic drugs. Tachyphylaxis to repeated injections is characteristic of these chemicals, presumably because of decreased availability of releasable stores of histamine. Endogenous substances that provoke histamine release and may be involved in physiological release mechanisms include bradykinin and stronger histamine releasing substances like kallidin and substance P. Cellular reactions to many venoms (e.g., wasp venom) and toxins also involve histamine release.

Physical Injury

When the skin is scratched or pricked, the characteristic redness and urtication that result are due to histamine. This response is quite pronounced in humans. Dermal reactions to severe cold or heat stress likewise depend on histamine liberated by local mast cells. Physical injury of virtually any type sufficiently intense to damage the cells will evoke release of histamine.

Histamine Receptors

The four histamine receptors are named in the order of their discovery: histamine H_1 receptor (H_1R) to H_4 receptor (H_4R) (Table 19.1). Classic pharmacology studies led to the discovery of H_1R, H_2R, and H_3R (Akdis and Simmons, 2006). Decades later their genetic structure was decoded and they were identified as belonging to membrane-bound G-protein coupled receptors. The most recently discovered, H_4R, was described in parallel to the cloning of the DNA sequence in 2000 (Oda et al., 2000).

H_1R couples to $G_{q/11}$ and activates the PLC-IP_3-Ca^{2+} pathway. Receptor expression is found in the smooth muscles of the respiratory, gastrointestinal, and urogenital tracts. It has also been identified in neuronal tissue including brain, spinal ganglia, and spinal cord (Akdis and Simmons, 2006). Additionally, H_1R is found on immune cells like T lymphocytes, dendritic cells, and endothelial cells. Activation of H_1R causes contraction of smooth muscle, vasodilation of arterioles and capillaries, increased vessel permeability, and stimulation of

Table 19.1 Characterization of histamine receptors in terms of tissue distribution, signal transduction, and physiological and pathophysiological function. Source: Adapted from Baumer and Rossbach, 2010. Reproduced with permission of John Wiley & Sons.

Receptor	H_1R	H_2R	H_3R	H_4R
Expression tissue	Smooth muscle in respiratory, gastrointestinal, uregenital tracts and vessels, nerve cells, hepatocytes, endothelial and epithelial cells, neutrophils, eosinophils, monocytes, dendritic cells, T and B cells	Parietal cells in gastric mucosa, nerve cells, smooth muscle in respiratory tract and vessels, hepatocytes, endothelial and epithelial cells, neutrophils, eosinophils, monocytes, dendritic cells, T and B cells	Histaminergic neurons, eosinophils, monocytes	Mast cells, basophils, eosinophils, monocytes, dendritic cell, T cells, sensory nerve cells, fibroblasts and keratinocytes, endocrine cells in gastrointestinal tract
Intracellulular signal cascade	Ca^{2+}↑, phospholipase C, NFκB	cAMP ↑, adenylate cyclase, c-Fos, c-Jun, PKC	cAMP ↓, Ca^{2+} ↑, MAP kinase	cAMP↓, Ca^{2+} ↑, AP-1↑
G-protein	$G_{q/11}$	$G_{\alpha s}$	$G_{i/o}$	$G_{i/o}$
Physiological function	Contraction of smooth muscle, increase of capillary permeability, mediation of itch, sleep–wake cycle	Glandular secretion, relaxation of smooth muscle	Regulation of sleep and food intake, cognition	Chemotaxis/ cytokine/ chemokine secretion by immune cells, mediation of itch
Pathological relevance	Immediate hypersensitivity	Acid-induced gastritis, gastrointestinal ulcers	Cognitive disorders, obesity	Inflammation, pruritus
Selective agonists	*N*-methylhistaprodifen	Amthamine, impromidine	Immethridine	ST 1006
Selective aantagonists	(+)-Chlorpheniramine	Cimetidine, Ranitidine	Pitolisant	JNJ7777120

afferent nerve endings. The effects, reddening of the skin, edema, and pruritus, are the classic symptoms of an allergic reaction. In addition to these peripheral effects, H_1R plays a pivotal role in the regulation of neuronal processes like food ingestion, the sleep–wake cycle, and triggering vomiting.

H_2R, comparably to H_1R, is widely distributed in peripheral tissues as well as in the central nervous system. The receptor is linked to G_s leading to activation of the adenylyl cyclase. One of the first actions discovered was the gastric acid production from parietal cells, which is largely mediated by H_2R (Black et al., 1972). However, beyond this effect, histamine acts via H_2R to relax smooth muscle, as has been shown in the respiratory tract, uterus, and vascular muscle. Stimulation of H_2R in the heart causes positive chronotropic and inotropic effects. Immunomodulatory action of histamine is also mediated via H_2R. Activation of H_2R influences the production of diverse cytokines in various types of cells such as monocytes, dendritic cells, and T cells, mainly to an antiinflammatory stage (Baumer and Rossbach, 2010).

H_3R couples to $G_{i/o}$ to inhibit adenylyl cyclase leading to decreased cyclic AMP. H_3R is pivotally expressed in the central nervous system (Lovenberg et al., 1999), where it is predominantly found in areas of the brain that are responsible for cognitive abilities. On the one hand, H_3R acts as an autoreceptor, regulating the synthesis and release of histamine from histaminergic neurons. On the other hand, this histamine receptor also regulates the release of various other neurotransmitters such as dopamine, serotonin, and acetylcholine from nonhistaminergic neurons (Sander et al., 2008). The wide distribution of H_3R in the central nervous system indicates a large list of possible indications for H_3R antagonists, including cognitive disorders, sleep disorders, obesity, Alzheimer's disease, and schizophrenia (Sander et al., 2008). The role of H_3R in inflammatory processes and in pruritus is also under investigation; an intradermal injection of a selective H_3R antagonist in mice can induce pruritus (Rossbach et al., 2011).

H_4R has the highest sequence homology to the H_3R. The H_4R is expressed on several cells of the immune system. Comparable to H_3R, it is coupled to $G_{i/o,}$ reducing, for example, forskolin-induced cAMP in cells. But activation of H_4R also induced Ca^{2+} influx in immune cells and sensory neurons. H_4R-expression occurs on numerous hematopoietic cells such as mast cells, basophils, eosinophils, different T cells, monocytes, macrophages, and dendritic cells (Thurmond et al., 2008). Expression of H_4R has also been shown on nerve cells of the dorsal root ganglia and in the spinal cord (Strakhova et al., 2009; Rossbach et al., 2011). H_4R seems to have a central function in modulating the immune response. H_4R influences cell activation, cell migration, and cytokine and chemokine production of various immune cells (Hofstra et al., 2003; Zampeli and Tiligada, 2009; Baumer and Rossbach, 2010). These findings have been corroborated by in vivo studies in which blockade of H_4R led to reduction of inflammation and pruritus (Thurmond et al., 2004, 2008; Cowden et al., 2010) and clinical trials with H_4R antagonists in humans for allergic-inflammatory diseases are currently under way (Kollmeier et al., 2014).

Pharmacological Effects

Histamine administered orally has essentially no effect because it is destroyed rapidly by the GI tract and liver. Intravenous histamine produces a spectrum of characteristic effects including smooth muscle contraction, hypotension, increased gastric secretion, and dermal reactions.

Difficulties are encountered when attempts are made to designate H_1- or H_2-receptor responsibility for each action of histamine. In some tissues, H_1 and H_2 receptors are complementary and subserve similar tissue responses. In contrast, distinct and even opposing functions of the two receptor types have been identified in some tissues. Species differences are formidable and in most cases await further study for classification. In the following paragraphs, only the more representative examples of H_1- or H_2-receptor involvement, when known, are discussed.

Cardiovascular System

The principal circulatory effects of histamine are dilation of terminal arterioles and other vessels of the microcirculation, edema formation caused by increased capillary permeability, and contraction of large arteries and veins. Relative dominance of the actions varies in different species so that net circulatory response to histamine changes as the zoological scale is ascended; for example, arterioles are contracted strongly by histamine in rodents, less so in cats, and actually are dilated in dogs, nonhuman primates, and humans.

In rabbits, histamine is a pressor agent as a result of pronounced constriction of large blood vessels. This constrictor activity is feeble in carnivores where vasodilation of the microcirculation dominates instead. Thus the blood pressure response to histamine in cats, dogs, and primates is hypotension caused by a sharp fall in peripheral vascular resistance. The fall in blood pressure is dose dependent but is usually short lived because of compensatory reflexes and inactivation of histamine.

The striking effects of histamine on the microcirculation can be demonstrated quite convincingly in the human subject. When this agent is administered intradermally, a characteristic triple response is produced, which includes: localized redness at the injection site, developing within a few seconds and attaining maximal hue within a minute; localized edema fluid, forming a wheal in about 90 seconds; and diffuse redness or "flare," extending about 1 cm from the original red spot. The central redness and edema are from the dilation and increased permeability of local microcirculatory vessels (terminal arterioles, capillaries, and venules). The surrounding flush, which is accompanied by itching and perhaps pain, is due to dilation of neighboring arterioles brought about by a poorly understood axonal reflex mechanism. The triple response of human skin may be similar to manifestations of urticaria in animals. Intradermal injection of histamine leads to a consistent wheal and flare reaction also in dogs. As therapeutically relevant concentrations of cetirizine reduce wheal and erythema by more than 80%, it is likely that these reactions are mainly mediated by activation of H_1 receptor (Bizikova et al., 2008).

Cardiac effects of histamine are minimal when compared to vascular actions. In the intact animal, slight tachycardia is a common finding. This response is mainly secondary to baroreceptor reflexes activated by the depressor effect. In isolated heart muscle, histamine can elicit positive inotropic and chronotropic effects that are produced partly by release of norepinephrine from nerve endings and also to direct activation of H_2 receptors in the heart muscle. There is some evidence that in vivo cardiac responses to histamine injection may partially reflect activation of cardiac H_2 receptors.

Nonvascular Smooth Muscle

Histamine contracts bronchial smooth muscle via H_1 receptors in numerous mammals including the guinea pig, cat, rabbit, dog, goat, calf, pig, horse, and human (Chand and Eyre, 1975; Mohammed et al., 1993; Vietmeier et al., 2007). Guinea pigs are exceptionally sensitive, and even minute doses of histamine can evoke bronchoconstriction leading to death. Humans with bronchial asthma likewise demonstrate increased sensitivity to bronchial effects of histamine and other bronchial smooth muscle stimulants. In contrast, histamine can mediate relaxation of respiratory smooth muscle in some species. Histamine-induced tracheal relaxation in cats involves both H_1 and H_2 receptors, while bronchial relaxation in sheep seems to be mediated by H_2 receptors (Hirschowitz, 1979).

Relaxation of the rat uterus by histamine is mediated by H_2 receptors, but uterine muscle of other species is generally contracted by histamine. Responses of intestinal muscle also vary with species and region, but the classic effect is a contractile response caused by H_1 receptors.

Peripheral Nerve Endings

In humans, nonhuman primates, and mice, intradermal injection of histamine induces itch by stimulation C-type nerve fibers (Johanek et al., 2007). Interestingly, dogs show low and very inconsistent itch behavior after intradermal injection of histamine (Carr et al., 2009). In mice and humans it has been shown that both H_1 and H_4 receptor antagonists reduce histamine induced itch, indicating also a central role for H_4R in histamine-dependent pruritus (Kollmeier et al., 2014).

Exocrine Glands

The following exocrine glands are listed in descending order of response to histamine: gastric, salivary, pancreatic, bronchial, and lacrimal. Gastric secretion of hydrochloric acid and, to a lesser degree, pepsinogen is unquestionably the most important; this response is mediated by H_2 receptors.

Medical Use

There is no direct medical use of histamine. It is used as a diagnostic agent to assess bronchial hypersensitivity in humans patients and it is serves as a positive control in humans and dogs during allergy skin testing.

Antihistamines

Although the pharmacological effects of histamine can be antagonized by several types of drugs, the term *antihistamine* is restricted to agents that act on histamine receptors.

Agents such as catecholamines and xanthines exhibit pharmacological activities that are, among other things, antagonistic to actions of histamine. However, these opposing actions are mediated by different receptors and cellular pathways; they represent physiological antagonism.

Development

Bovet and Staub (1937), of the Pasteur Institute in Paris, first demonstrated that two phenolic esters possessed antihistaminic activity. One of these compounds, 929F (thymoxyethyldiethylamine), protected guinea pigs against several lethal doses of histamine. Although the original drugs were too toxic for therapeutic use, their discovery led to development of many modern antihistamines. Such compounds are now referred to as H_1 and H_2 antihistamines, based on the previously described differentiation of histamine receptors into H_1 and H_2 subtypes (Ash and Schild, 1966; Black et al., 1972). Currently, there are also H_3 and H_4 antihistamines in clinical trials but no substance is close to marketing and only very few data exist for species relevant to veterinary science.

H_1 Receptor Antagonists

H_1 receptor antagonists are classified as inverse agonists, rather than histamine antagonists (Simons, 2004), as they reduce constitutive activity of the receptor and compete with histamine. The binding of the natural ligand histamine induces a fully active conformation, whereas antihistamine binding yields an inactive conformation. However, the term *histamine antagonists* is still often used in veterinary literature.

The H_1 antagonists have been divided into the first-generation antihistamines (e.g., chlorpheniramine, diphenhydramine, and hydroxyzine) and the second-generation antihistamines (e.g., cetirizine, loratadine, desloratadine, and fexofenadine). The second-generation antihistamines are the newer, nonsedating antihistamines. This group includes most of the newer antihistamines introduced since 1981. Some of these drugs are related: cetirizine is a metabolite of hydroxyzine and desloratadine is a metabolite of loratadine.

The primary difference between the first- and second-generation antihistamines is that the second-generation antihistamines lack the antimuscarinic properties and do not cross the blood–brain barrier as easily as first-generation antihistamines. Therefore these drugs lack the central nervous system side effects, particularly sedation, that is common with the first-generation antihistamines. The effects of each group of antihistamines will be discussed in more detail below.

Chemistry

Some of the more frequently used H_1 antihistamines are listed in Table 19.2. The chemical structure of nearly all the H_1 antihistaminic drugs can be depicted by the structural formula shown in Figure 19.2. Like histamine, many H_1 antagonists contain a substituted ethylamine (CH_2CH_2N) moiety. This moiety is thought to be the molecular component necessary for competition with histamine for specific cell receptors.

Three types of H_1 antihistamines are known, in which the element X (as depicted in Figure 19.2) is nitrogen, oxygen, or carbon. The X represents a nitrogen for the ethylenediamine class (e.g., pyrilamine, Neoantergan®), oxygen for the ethanolamine class (e.g., diphenhydramine, Benadryl®), and carbon for the alkylamine class (e.g., chlorpheniramine, Teldrin®). The fourth class of antihistamines contains a piperazine in place of the conventional ethylenediamine linkage (e.g., cyclizine, Marezine®). The representative of the fifth class (e.g., promethazine, Phenergan®) is not related directly to the

Table 19.2 Preparations and doses of some H_1 antihistamines in veterinary use

Drug class	Drug name	Brand name	Dose in animals
First-generation antihistamines (H_1 antagonists)			
Alkylamine	Chlorpheniramine	Chlor-Trimeton	Dog: 4–8 mg/dog to a maximum of 0.5 mg/kg q 8–12 h Cat: 2–4 mg/cat, q 12 h
Ethanolamine	Diphenhydramine	Benadryl	2–4 mg/kg q 8–12 h
Ethanolamine	Clemastine	Tavist and generic	0.05–0.1 mg/kg q 12 h – poor oral absorption (dogs/horses)
Piperazine	Hydroxyzine	Atarax	0.5–2 mg/kg q 8 h
Phenothiazine	Trimeprazine	Temaril, Panectyl	0.5 mg/kg q 12 h
Second-generation antihistamines (H_1 antagonists)			
	Fexofenadine	Allegra	Not established
	Loratadine	Claratin (nonprescription OTC)	Not established
	Cetirizine	Zyrtec	Horse: 0.4 mg/kg q 12 h Dog: 2 mg/kg q 12 h Cat: 1 mg/kg daily
	Desloratadine	Clarinex	Not established

previous drugs; it is a phenothiazine derivative. The sixth class comprises the piperidines terfenadine and fexofenadine; these agents have aromatic ring moieties on either end of the ethylamine chain. Terfenadine is no longer used due to cardiotoxicity (QT interval prolongation); fexofenadine lacks this side effect. These different chemical substitutions influence the potency of H_1-antihistaminic action as well as their side effects.

Pharmacological Effects

Pharmacokinetic information for the antihistaminics of the H_1 subtype in different species is sparse. Chlorpheniramine is well absorbed in dogs, with a short time to peak absorption after oral administration and a half-life of 24 hours. Some are absorbed poorly (clemastine) (Hansson et al., 2004), while others appear to be well absorbed, but rapidly converted to a metabolite (hydroxyzine) (Bizikova et al., 2008). There are little data available for horses or ruminants. When clemastine was administered to horses (Törneke et al., 2003), there was a rapid decline in plasma concentrations. Oral absorption was only 3% at a dose of 0.2 mg/kg. There has been some PK/PD modeling in horses with oral administration of cetirizine. A dose of 0.4 mg/kg cetirizine BID resulted in nearly 70% wheal inhibition induced by histamine; the half-life of cetirizine was roughly 6 hours in this setting (Olsén et al., 2008).

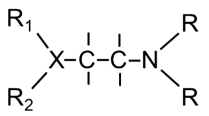

Figure 19.2 General formula of most H_1 antihistaminic agents.

In species for which oral absorption occurs, effects are usually expected within 20–45 minutes, and the duration of action ranges from 3 to 12 hours (Table 19.2). IV administration elicits immediate effects, but this route is not used except for treatment of acute anaphylaxis (for which epinephrine is the preferred treatment of choice). Rapid IV injection can produce stimulation of the CNS and other side effects. The intramuscular route rarely gives rise to side effects and is more commonly used than IV administration. Topical application may be suitable in certain skin conditions, but antihistamines can be skin sensitizing, particularly during long-term use (Robinson and Cruze, 1996).

Antihistamines act as inverse agonists to stabilize a low constitutive activity of the histamine H_1 receptors in the tissue cells; their binding to the cell receptors evokes no direct cellular action. This mechanism of action is based on quantitative considerations; therefore, histamine in excess may displace antihistaminics. Generally, antihistaminics are more effective against exogenously administered histamine than against endogenously released histamine. They are also more effective in preventing actions of histamine than in reversing them (Simons, 2003).

Therapeutic Uses

Generally, H_1 antihistaminics are useful in countering action of histamine on bronchial, intestinal, uterine, and vascular smooth muscle (Gelfand et al., 2004). They antagonize both the vasoconstrictor effects of histamine and the more important vasodilator effects, as well as the increase in capillary permeability produced by this agent. These antihistaminic effects counteract urticaria, wheal formation, and other types of edema formation in response to injury, antigens, allergens, or histamine-liberating drugs. H_1 antihistaminics also suppress itching and flare in humans and greatly reduce itching associated with allergic reaction. In addition to the traditional effect on histamine response, these drugs may have other antiinflammatory/immunomodulatory effects, including decrease of the release of inflammatory mediators from inflammatory cells such as mast cells (Simons and Simons, 1994; Walsh, 2005). The relevance of this activity to clinical response is not known, nor is it known if this occurs at clinically achieved plasma concentrations. H_1 antihistaminics only partially antagonize histamine-induced arterial hypotension because portions of this response are associated with H_2 receptors. The H_1 antagonists do not block the stimulant effect of histamine on gastric secretion, which is an H_2-dependent function. Importantly, neither H_1 nor H_2 antihistamines prevent histamine release; some antihistaminics rather seem to possess histamine-liberating properties.

Veterinary Medicine

H_1 antihistaminics are frequently used in animals and representative doses are given in Table 19.2; however, as mentioned above, there are only few PK/PD data available for H_1 antagonists and most data are extrapolated from use in human medicine. Clinically, H_1 antagonists are used to prevent reactions from endogenous histamine as a response to certain allergic disorders and anaphylactic syndromes. However, the clinician must be aware that autacoids other than histamine also play important roles in allergy-anaphylaxis disorders. During mast cell degranulation, apart from histamine, the following mediators are released and secreted: serotonin, dopamine, kinins, prostaglandins, leukotrienes, platelet activating factor, complement, and several cytokines and chemokines. Thus it is not surprising that antihistamines alone are often ineffective in treating allergic-type reactions in animals.

Clinical signs of allergy vary among different species. The most frequently observed signs are restlessness, anorexia, yawning, salivation, lacrimation, nasal discharge, coughing, edema, urticaria, pruritus, eczema, necrosis, hemorrhage, inflammation of the mucous membranes and eyes, contraction of smooth muscle (bronchoconstriction), and cardiovascular disturbances. In acute or delayed anaphylaxis, clinical signs occur quickly and, if not treated, are followed by collapse and death in minutes.

Treatment of allergic conditions (e.g., atopic dermatitis) consists of further avoidance of allergens, hyposensitization, corticosteroids, cyclosporine, oclacitinib, and, rather as add on therapy, the administration of H_1 antihistaminics.

Treatment of Pruritus

The most extensively studied clinical problem in veterinary medicine for which antihistamines have been used is pruritus associated with atopic dermatitis (Papich, 2000; DeBoer and Griffin, 2001). Evidence-based reviews were published by Olivry et al. (2003, 2010, 2013). In some reports the incidence of response is approximately the same as a placebo. Several of the reported studies are uncontrolled, or the studies are not published in reviewed references. Nevertheless, these drugs are used by dermatologists to decrease the reliance on corticosteroids or used in conjunction with other antiinflammatory medications. As reported by Zur and colleagues (2002), dermatologists will often try three to five antihistamines in 2-week trials to find the one that is most effective for a patient. As expected, because of these individual variations, there also are a variety of results reported. Zur and colleagues (2002) evaluated hydroxyzine, diphenhydramine, chlorpheniramine, and clemastine in a retrospective study. Overall, 54% of the dogs had a good to moderate response. In that study, diphenhydramine and hydroxyzine were the most often used, and the most often effective. The second-generation antihistamine fexofenadine was tested in a small clinical trial (15 dogs) in the comparably high dose of 18 mg/kg over 6 weeks. At the end of the study, 80% of the dogs showed a reduction in pruritus >50% (Olivry and Bizikova, 2013). Although encouraging, larger studies are needed to test the efficacy and safety of fexofenadine for the treatment of atopic itch.

Clemastine (Tavist), an ethanolamine antihistamine, has a poor oral availability in dogs (Hansson et al., 2004) and horses (3%) and it is thus not recommended to be administered orally. Chlorpheniramine, diphenhydramine, and hydroxyzine may be effective in some dogs (Scott and Buerger, 1988). Trimeprazine, a phenothiazine derivative with antihistamine effects, has little effect on its own, but is among the most effective drugs when combined with a corticosteroid (prednisolone).

Cetirizine, one of the second-generation drugs, is an active metabolite of hydroxyzine. Some veterinarians have claimed some success, but it was modest at 1 mg/kg/day (18% response). In a study in dogs (Bizikova et al., 2008), it was shown that hydroxyzine (a first-generation antihistamine) was rapidly converted to cetirizine (a second-generation antihistamine) in dogs. This conversion was rapid regardless of the route (IV or oral at a dose of approximately 2 mg/kg). Moreover, the observed reaction to histamine was attributed to the plasma concentrations of cetirizine, not hydroxyzine. Therefore, hydroxyzine – one of the most popular antihistamines used in dogs – appears to act as a prodrug. A controlled blinded cross over study comparing dimetinden (0.1 mg/kg) with the combination of 0.07 mg/kg chlorpheniramine and 2 mg/kg hydroxyzine in dogs suffering from atopic dermatitis confirm the overall moderate effect of H_1 antihistamines on pruritus as well as on skin lesions (Eichenseer et al., 2013). These results are corroborated by findings in a dog model of atopic dermatitis; the determination of histamine after allergen challenge in atopic dogs sensitized to house dust mite antigen revealed that only within the first 30 to 45 minutes, there was a transient increase of histamine in the skin. Both, histamine H_1 and H_4 receptor antagonists did not prevent the generation of acute skin lesions in this dog model (Baumer et al., 2011). An effect on itch, however, was not determined in this study and awaits further investigation.

Chlorpheniramine and clemastine also have been reported to reduce pruritus in cats (Miller and Scott, 1990). The effects or pharmacokinetics of antihistamines in this species have not been studied as much as in dogs. In one study, cetirizine (ca. 1 mg/kg) was well absorbed in cats and produced plasma concentrations that were above the levels considered therapeutic in humans.

Some studies have shown that combinations of antihistamines with fatty acids or other drugs may improve efficacy. There may be a synergistic effect of antihistamines (e.g., clemastine, chlorpheniramine) in combination with n-3/n-6 fatty acids (Paradis et al., 1991a), and evidence for a synergistic effect with fatty acids and corticosteroids. When antihistamines were combined with corticosteroids in one report, the effective dose of prednisone was lowered (30% reduction) (Paradis et al., 1991b). In a study in dogs, a combination of a fatty acid product plus clemastine was more effective (43% response) than either drug used alone (Paradis et al., 1991a). In cats, chlorpheniramine plus fatty acids were more effective than either drug used alone. Despite these positive results, the study by Zur et al. (2002) did not show combinations to be helpful.

There are only few data on antihistamine treatment of horses suffering from insect bite hypersensitivity (“sweet itch”). Cetirizine at the dose of 0.4 mg/kg BID did not show any effect on dermatitis score (Olsén et al., 2011).

Treatment of Anaphylaxis

Anaphylactic syndrome requires emergency treatment because it progresses rapidly to irreversible cardiovascular collapse. The drug of choice is epinephrine; this catecholamine does not directly inhibit mediators of anaphylaxis but reverses their effects. Thus epinephrine acts as a physiological antagonist (see Chapter 7).

Treatment of Respiratory Disorders

The use of antihistamines for treatment of inflammatory airway disease and asthma has been of only minor benefit, suggesting the important contribution of other inflammatory mediators in this disease.

Cetirizine produced a protection against bronchoconstriction in humans affected with mild asthma during histamine challenge and may have other antiinflammatory properties (Walsh, 2005). However, in sensitized asthmatic cats, cetirizine at a dose that produces plasma concentrations considered effective in people (5 mg per cat every 12 h) was ineffective in attenuating the infiltration of inflammatory eosinophils in airways or in altering other immune variables (Schooley et al., 2007). These authors recommended that cetirizine (and perhaps other antihistamines) should not be considered as sole therapy for asthma in cats. Antihistamines also are considered to be of value in treatment of bovine asthma (pulmonary emphysema). Chapter 48 presents a more detailed discussion of drugs that affect the respiratory system.

Treatment of Other Conditions with Antihistamines

Nonallergic but suspected histamine-related phenomena, which in empirical experience respond to antihistaminic therapy in animals, include many pathological conditions. Those in which H_1 antihistaminics are reported to be of therapeutic value are: urticaria, various types of dermatitis, moist eczema, acute eczematous otitis, insect stings, nutritional types of laminitis, pregnancy laminitis, paroxysmal myoglobinuria or azoturia, periodic ophthalmia, and pulmonary emphysema in horses. In cattle, antihistamines also are considered to be of value in treatment of some types of bloat and acetonemia in ruminants, acute septic and gangrenous mastitis, septic metritis and retained placenta, pregnancy toxemia, and gut edema of pigs. Treatment of vestibular stimulation (motion sickness) in animals has been variable. Although these drugs are sold over-the-counter for this purpose for humans, they appear to be less effective for vestibular vomiting in animals (Yates et al., 1998).

Side Effects and Interactions

In recommended doses H_1 antihistaminics are relatively nontoxic. Side effects of clinical importance for the H_1 blockers include sedation or CNS excitement, GI disturbances, parasympatholytic action, local anesthetic properties, allergenic properties, and teratogenic effects. In therapeutic doses, the first generation H_1 antihistaminics elicit a sedative effect expressed by drowsiness or ataxia. The newer, second-generation antihistamines are more popular in people because they are considered nonsedating. Some dermatologists have acknowledged that the effect on reducing pruritus in dogs after administration of first-generation antihistamines may be partially attributed to the sedation produced (DeBoer and Griffin, 2001; Olivry et al., 2003, 2010). Despite an assumption that first-generation antihistamines cause sedation in animals, a study of diphenhydramine in dogs indicated that this assumption may be incorrect. When dogs were administered doses of 2, 4, or 8 mg/kg IM compared to acepromazine or saline, the dogs receiving acepromazine showed significant sedation but sedation in dogs receiving diphenhydramine was no different from saline (Hofmeister and Egger, 2005).

In much higher doses they produce irritability, convulsions, hyperpyrexia, and even death. Intestinal disorders include anorexia, nausea, vomiting, constipation, or diarrhea when antihistamines are administered orally for a prolonged period. The anticholinergic effects are expressed by dry mouth, pupillary dilation, blurred vision, and tachycardia. Local anesthetic properties may be of value when these agents are used as antipruritic drugs in topical application. The teratogenic effects of certain of these agents suggest caution in their use during pregnancy.

The newer, second-generation H_1 antihistamines – fexofenadine, cetirizine, loratadine – are largely excluded from the CNS when given in therapeutic doses because they are better substrates for the P-glycoprotein (P-gp) membrane pump that is an important component of the blood–brain barrier (Sun et al., 2003; Janssens and Howart, 1993; Meeves and Appajosyula, 2003). Their lack of sedation as a side effect is a distinct advantage in human medicine. Study is needed in veterinary medicine to determine their clinical efficacy and whether lack of sedation is an important attribute in animals (Miller et al., 1989).

H_2 Receptor Antagonists

H_2 antagonists block the gastric stimulating effects of histamine as well as other actions of histamine that have been defined as H_2-receptor dependent (e.g., stimulation of rat uterus, cardiac excitatory effects, and some vascular effects).

The H_2 antihistamines differ from the H_1 blockers in their chemistry, pharmacokinetics, and pharmacodynamics. The imidazole ring structure of histamine is modified extensively or replaced by other substituents in the H_1 antagonists. In the H_2-blocking agents, however, the side chain is modified extensively, while the imidazole moiety is preserved. In contrast to the H_1 antihistaminics, the H_2 antagonists are somewhat less lipid soluble and do not effectively penetrate the blood–brain barrier. Hence, the H_2 antagonists do not cause sedation, a prominent side effect of most of their H_1 counterparts.

Burimamide was the first H_2 antagonist, but it was absorbed too poorly to be effective after oral administration. Cimetidine, ranitidine, and famotidine are some of the newer H_2 blockers used extensively in prevention and treatment of gastric ulceration in humans and animals. (H_2-blocking drugs used to treat stomach disorders are discussed in more detail in Chapter 46.)

Serotonin

Rapport et al. (1948) isolated a vasoconstrictor substance from serum and gave it the name serotonin; it was subsequently identified as 5-hydroxytryptamine (5-HT). Another group of researchers studying histochemical properties of the intestinal mucosa discovered an active agent in enterochromaffin cells and gave it the name enteramine (Erspamer and Asero, 1952). After discovery of 5-HT in blood, it was soon confirmed that enteramine and 5-HT were the same chemical structure.

Following discovery of 5-HT and multiple types of 5-HT receptors in the CNS, it was soon discovered that drugs affecting 5-HT in the brain had beneficial CNS actions including modification of behavior, antidepressant actions, and antianxiety effects (Sanders-Bush and Mayer, 2006). Drugs that modulate 5-HT action are being used increasingly in veterinary medicine in attempts to control behavioral problems in animals, and modify gastrointestinal motility (for additional information see Chapters 18 and 46) (Crowell-Davis and Murray, 2006; Mohammad-Zadeh et al., 2008).

Sources, Synthesis, and Metabolism of Serotonin

5-HT is synthesized from dietary tryptophan in a two-stage chemical reaction (Walther et al., 2003). First, tryptophan is hydroxylated by the enzyme tryptophan 5-hydroxylase to give 5-hydroxytryptophan (5-HTP). The latter is then decarboxylated to yield 5-HT (serotonin), as shown in Figure 19.3.

Like histamine, 5-HT is widely distributed in animals and plants (Sanders-Bush and Mayer, 2006). It occurs in high concentration in some fruits such as bananas, pineapples, and plums; it also is present in stings (common stinging nettle) and venoms. Endogenous 5-HT is synthesized from about 1% of the dietary tryptophan. It is formed and localized in three essential pools: enterochromaffin cells of the intestine, select neurons in the CNS, and mast cells of rodents (rats, mice, hamsters), and possibly other species (conflicting results), along with histamine and heparin. Although 5-HT is concentrated in blood platelets, it is not synthesized there because of lack of decarboxylase. It appears to be bound within cytoplasmic granules and is also continually produced and destroyed in the pool of the intestine and brain. In platelets it appears to be released only upon their destruction.

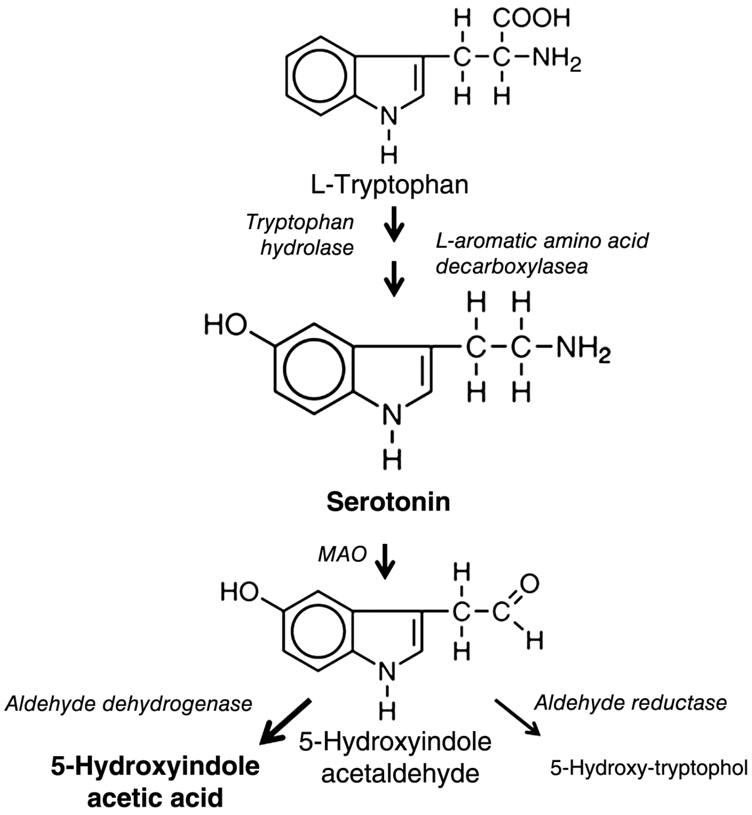

Figure 19.3 Synthesis and metabolism of serotonin. Serotonin is synthesized from L-tryptophan by a two-step pathway via 5-hydroxytryptophan. The main route of metabolism of serotonin involves oxidative deamination by monoamine oxidase (MAO). Via an aldehyde intermediate, the main metabolite is 5-hydroxyendole acetic acid. The reduction to 5-hydroxytryptophol is normally insignificant.

Most 5-HT is metabolized by oxidative deamination to form 5-hydroxyindoleacetic acid (5-HIAA); the enzyme catalyzing this reaction is monoamine oxidase (Figure 19.3). The end product of metabolism, 5-HIAA, is excreted in urine. However, in the pineal gland, *N*-acetylation and 5-methylation of 5-HT form the hormone melatonin.

Neuronal Uptake of 5-HT

After 5-HT is synthesized, it is stored within secretory granules in the axon terminal from which it is released by exocytosis upon arrival of the action potential. The action of intrasynaptic 5-HT is terminated in large part by a neuronal membrane uptake transporter localized in the axon terminals of the 5-HT neurons. This reuptake by the neurons diminishes 5-HT availability at the postsynaptic 5-HT receptors of the effector cells, thereby regulating the duration and intensity of action of 5-HT. Pharmacological blockade of the 5-HT uptake mechanism thereby prolongs the availability and action of 5-HT at its receptors on the effector cells. The action of 5-HT is subsequently intensified. The 5-HT transporter (SERT) has been cloned; it is selective for 5-HT and distinctly different from the intraneuronal storage granule uptake mechanism.

The 5-HT neuronal uptake transporter has become important to neuropharmacology in both human and veterinary medicine (Crowell-Davis and Murray, 2006). Drugs that inhibit 5-HT uptake, known as selective serotonin reuptake inhibitors (SSRIs or SRIs), are used extensively to treat depression and other neurological disorders, alone or jointly with drugs that inhibit norepinephrine reuptake into noradrenergic neurons (Sanders-Bush and Mayer, 2006). In veterinary medicine SSRIs are used in attempts to control various behavioral disorders in small animals (see Chapter 18). Their clinical use is becoming more common in animals owing to the increased recognition by veterinarians that treatment of behavioral problems in dogs and cats can often be facilitated by drugs that enhance the action of CNS neurotransmitters such as 5-HT, norepinephrine, and dopamine (Crowell-Davis and Murray, 2006). The SSRIs include citalopram (Celexa®), sertraline (Zoloft®), fluoxetine (Prozac®, Reconcile®), and paroxetine (Paxil®). Of these, only fluoxetine has been registered for dogs, although the others have been used off-label. Clomipramine (Clomicalm®, Anafranil®) is a tricyclic antidepressant that has been approved for the treatment of separation anxiety in dogs; it inhibits the neuronal reuptake of both 5-HT and, to a far lesser extent, norepinephrine (Crowell-Davis and Murray, 2006).

Subtypes of 5-HT Receptors

Because of the wide variety of pharmacological actions produced by 5-HT, multiple types of receptors seemed likely (Bonasera and Tecott, 2000). Subsequent investigations, including cloning of receptor cDNAs, confirmed that there was indeed an impressive variety of 5-HT receptor families comprising at least seven major receptor types and several respective subtypes. Although their full spectrum of physiological effects and pharmacodynamic actions has only been partially characterized, most of the 5-HT receptors (except 5-HT_3) are believed to be typical G-protein coupled receptor complexes (Sanders-Bush and Mayer, 2006).

The seven major 5-HT receptor families are designated as 5-HT_1 through 5-HT_7. The 5-HT_1 family has five members designated 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1D}, 5-HT_{1E}, and 5-HT_{1F}. All five 5-HT_1 receptors inhibit adenylyl cyclase, thereby reducing intracellular concentrations of the cyclic nucleotide cAMP. The 5-HT_{1A} receptor also inhibits a voltage-gated Ca^{2+} channel, but activates a receptor-coupled K^+ channel. The 5-HT_1 receptors in the CNS subserve a variety of excitatory and inhibitory neurotransmitter actions in different regions of the brain. 5-HT_{1A}-receptor agonists include buspirone, gepirone, and ipsapirone; these agents comprise a new class of antianxiety drugs in human medicine. Some experience exists in cats with buspirone (see Chapter 18). The 5-HT_{1D} agonist sumatriptan constricts intracranial blood vessels, an action that helps reduce migraine headaches in humans. The 5-HT_{1B} selective agonists seem to share this action.

Three subtypes of 5-HT_2 receptors (5-HT_{2A-C}) also have been identified; they activate the phospholipase C-diacylglycerol-inositol triphosphate triade, culminating in mobilization of intracellular Ca^{2+} stores (Janssen, 1983; Gray and Roth, 2001).

Unlike the other 5-HT receptor types, the 5-HT_3 receptor is the only known monoamine receptor believed to serve as a ligand-operated ion channel (Derkach et al., 1989), somewhat analogous to the nicotinic cholinergic receptor. The 5-HT_3 receptor promotes a rapidly desensitizing depolarization, presumably due to the efflux of K^+ through its opened channels. The 5-HT_3 receptors are present both in the CNS and peripheral neurons, with one action associated with coordination of the emetic response.

The 5-HT_4 receptors are located in various regions of the CNS and the gastrointestinal tract (Hegde and Eglen, 1996), where they are coupled to activation of adenyl cyclase through the excitatory G-protein (G_S). The actions and effects of the 5-HT_5, 5-HT_6, and 5-HT_7 receptor types remain to be clearly defined (Sanders-Bush and Hazelwood, 2011). Data indicate a role of 5-HT_6 in cognitive functions (Ramírez, 2013); the 5-HT_7 is also involved in learning and memory but additionally plays a role in thermoregulation and influences the circadian rhythm (Gellynck et al., 2013).

Pharmacological Effects

5-HT exerts multiple actions with great variation in different species, reflecting its multiple locations and plethora of receptor types. Its essential effects are on smooth muscle and central and peripheral nerves, including afferent nerve endings. Given orally, it is quickly degraded and produces no effect.

Rapid IV injection of 5-HT produces a triphasic response: an initial fall of systemic arterial pressure accompanied by paradoxical bradycardia, caused mainly by activation of 5-HT_3 receptors on vagus nerve endings (Bezold-Jarisch effect); a short period of pressor effect (similar to epinephrine effect); and a prolonged fall in systemic blood pressure attributed to a vasodilator effect in the vascular bed of skeletal muscle. 5-HT also causes a fall in pulmonary arterial pressure (pulmonary depressor reflex). A continuous infusion of 5-HT, which most closely resembles endogenous release of this agent, causes a prolonged fall in arterial pressure as a result of vascular bed dilation. Only in rodents does this agent increase small vessel permeability similar to effects of histamine.

The nonvascular smooth muscle of the bronchi and intestines is stimulated by 5-HT. In the intestine, as a local hormone, 5-HT initiates a peristaltic reflex in response to local stimulation. Among the receptors, 5-HT_3 is inhibitory, and 5-HT_4 is excitatory. Several drugs affect these receptors: cisapride is an agonist for 5-HT_4; metoclopramide is an agonist for 5-HT_4 and (weak) antagonist for 5-HT_3. Ondansetron, which is used as an antiemetic agent has modest prokinetic activity via 5-HT_3 antagonism. Tegaserod and prucalopride, two newer drugs for which only experimental data exist for animals, act as 5-HT_4 agonists with colon-specific effects. The clinical use of these drugs to affect GI motility is discussed in more detail in Chapter 46.

When 5-HT is injected, it has no effect on the brain or spinal cord because it is strongly polar and cannot effectively cross the blood–brain barrier. However, 5-hydroxytryptophan (5-HTP) can penetrate into the brain and be decarboxylated to 5-HT; this may produce behavioral changes. 5-HT can also stimulate afferent nerve endings, ganglion cells, and adrenal medullary cells. 5-HT can also stimulate vomiting because the chemoreceptor trigger zone that stimulates vomiting in the area postrema is outside of the blood–brain barrier.

Role in Physiological and Pathological Processes

The finding that 5-HT is present in the CNS, the hypothalamus, and other areas led to identification of its role as a central neurotransmitter. 5-HT influences sleep, sensory perception, cognition, motor activity, appetite, intestinal motility, temperature regulation, and mood and behavior (Crowell-Davis and Murray, 2006). An excess of this agent brings about CNS stimulation and mood elevation, while a deficiency produces mood depression. The addition of SSRIs as a separate class of antidepressant drugs followed the realization that increasing the concentration of 5-HT at its CNS receptor sites could improve mood (see Chapter 18). In the peripheral nervous system, 5-HT is involved in the perception of itch and pain (Liu and Ji, 2013). The role of 5-HT in platelets is related to the mechanism of hemostasis via both vasoconstriction and platelet aggregation.

5-HT Agonists and Antagonists

Because several classes and subclasses of 5-HT receptors have been identified (Derkach et al., 1989), it is not surprising that 5-HT receptor antagonists and agonists comprise a broad group of chemically unrelated compounds. Neural effects of 5-HT in smooth muscle of the digestive tract are antagonized by morphine, atropine, and cocaine; the direct effects on smooth muscle are antagonized by phenoxybenzamine. Chlorpromazine and phenoxybenzamine are weak 5-HT receptor blocking agents. Reserpine depletes serotonin (and other monoamines) in the synapses by inhibition of the vesicular monoamine transporter. Cyproheptadine acts on 5-HT_{1A} receptors, but it is also a H_1 antihistamine. Ketanserin is a 5-HT antagonist that acts preferentially at the 5-HT_{2A} receptor subtype without significant action at the 5-HT_1 or 5-HT_3 receptors. It is also a weak H_1 antihistamine and α-adrenergic blocker.

The areas in which drugs have been most widely investigated in veterinary medicine are the use of cyproheptadine in animals to treat inflammatory conditions and to stimulate appetite, the use of serotonin antagonists to treat vomiting, the use of serotonin agonists to stimulate gastrointestinal motility, and the use of drugs that modify serotonin CNS receptors or alter the reuptake of serotonin to treat behavioral disorders. The use for treating behavior disorders is discussed in detail in Chapter 18 and by Crowell-Davis and Murray (2006). The gastrointestinal uses of these drugs (agonists and antagonists) are discussed in Chapter 46.

Cyproheptadine

Cyproheptadine (Periactin®) is a serotonin antagonist and a modest antagonist for histamine (H_1). It has been used to treat inflammatory conditions of the skin (dermatitis) and the airways. It is also used as an appetite stimulant in cats, presumably by inhibiting serotonergic receptors in the hypothalamus that control satiety. Doses used to stimulate appetite in cats are in the range of 1 mg per cat orally once daily, to as high as 8 mg orally, every 8 hours. In cats the half-life is approximately 8 and 12 hours after IV and oral administration, respectively, suggesting that twice-daily administration would be sufficient in most cats (Norris et al., 1998). It was completely absorbed after oral administration (Norris et al., 1998).

Serotonin is reported to be one of the inflammatory mediators in the airways of animals – particularly the acute phase response of asthma. Therefore, serotonin antagonists such as cyproheptadine in cats should theoretically produce some benefit against smooth muscle constriction, vasodilation, increased vascular permeability, and inflammatory cell influx. Cyproheptadine attenuated the smooth muscle constriction induced by serotonin in airway smooth muscle from sensitized cats (Padrid et al., 1995). Cyproheptadine also has decreased airway reactivity to serotonin infusion in healthy cats (Reiche and Frey, 1983) and at a dose of 2 mg/kg orally q 12 h it decreased airway reactivity in cats with airway hyperresponsiveness (Reinero et al., 2005). However, in cats with experimentally induced asthma, cyproheptadine at a dose of 8 mg per cat orally produced limited results (Schooley et al., 2007). It did not decrease eosinophilic infiltration in the airways of treated cats. These authors suggested that perhaps mediators other than histamine and serotonin are responsible for airway responses in asthmatic cats and antihistamines and cetirizine as sole therapy will produce minimal benefit. Adverse effects in cats have not been studied, but side effects of aggression and stimulation have been observed. Additional discussion of drugs that affect the respiratory tract is included in Chapter 48.

Side Effects and Interactions

Side effects of 5-HT agonists and antagonists vary, mainly due to their specific receptor interaction or due to the site of action. Generally, care must be taken to avoid the “serotonin syndrome” (or serotonin toxicity), characterized by an excess of serotonin in the CNS. Clinical signs include nausea, vomiting, mydriasis, hypersalivation, and hyperthermia (Fitzgerald and Bronstein, 2013). Thus, combinations of SSRI, monoamine oxidase inhibitors, and tricyclic antidepressants should be avoided. For details of specific side effects of 5-HT agonists and antagonist please refer also to Chapters 18 and 46.

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20

Analgesic, Antiinflammatory, Antipyretic Drugs

Peter Lees

Scientific Literature

The published literature on nonsteroidal antiinflammatory drugs (NSAIDs) is voluminous in quantity but not always matched by quality; in amount, it is greater than any individual can read in a lifetime. For those wishing to rise to this challenge, the following chapters and reviews are recommended as a starting point: Vane, 1971; Warner et al., 1999; Flecknell and Waterman-Pearson, 2000; Lees et al., 2004a,b, 2015; Bergh and Budsberg, 2005; Robertson, 2005; Lascelles et al., 2005, 2007; Papich, 2007, 2008; Budsberg, 2009; Innes et al., 2010; Kukanich et al., 2012; Lees and Toutain, 2013). The reader is also referred to the USP monographs on individual drugs (Veterinary Medicine Expert Committee on Drug Information, 2004).

The Role of Eicosanoids in Inflammation and Mechanisms of Action of NSAIDs

Acute inflammation is characterized by the four cardinal signs of heat, redness, swelling, and pain, as first described by the Roman physician Celsus in the first century AD, to which Virchow added the fifth sign, loss of function, in the 19th century. Three of the cardinal signs involve the microcirculation, being caused by arteriolar dilatation and increased permeability of capillaries and postcapillary venules to protein, leading to formation of an inflammatory exudate rich in leukocytes. The infiltrate is first dominated by polymorphonuclear leukocytes and later by mononuclear cells, which transform to macrophages in the interstitial space. Leukocytes are the scavengers of the inflammatory process, engulfing microorganisms, particulate matter, and dead and dying cells, and releasing enzymes and chemicals, the mediators of inflammation. Inflammatory processes are driven by a wide range of mediators, synthesized de novo or released from cell storage sites, in a timed manner in both the early and resolution phases of acute inflammation and in chronic inflammation. Many mediators interact with other mediators by addition, antagonism, or synergism.

Arachidonic acid (AA), a 20-carbon ω-6 unsaturated fatty acid, plays a pivotal role in inflammation as the precursor of the eicosanoid group of mediators. AA is an esterified component of cell membrane phospholipid, released in tissue damage following the action of endogenous peptides, the lipocortins, which activate phospholipase A_2. There are several isoforms of phospholipase A_2 and it is the cytosolic 85-kDa isoform that normally supplies AA. AA serves as a substrate not only for prostaglandin (PG)H synthase, more commonly known as *cyclooxygenase (COX),* of which there are two isoforms COX-1 and COX-2, but also several lipoxygenases (LO), including 5-LO, 12-LO, and 15-LO. Each enzyme is a component of a cascade, in which the action of further enzymes leads to the formation of eicosanoids. They are described as autacoids, as they act locally at the site of synthesis. Moreover, they are short lived, so that continued effect depends on maintained synthesis and release. COX catalyses both the formation of PGG_2 and then PGG_2 conversion to PGH_2 via a peroxidase function.

At an early stage of acute inflammation, several PGs (including PGE_2, PGI_2, and PGD_2) are synthesized from AA, the product profile being determined by differing downstream enzymes. For example, cytosolic PGE synthase is mainly coupled with COX-1, whilst an inducible microsomal or membrane-associated perinuclear PGE synthase, regulated by cytokines and glucocorticoids, is coupled with COX-2 (Murakami et al., 1999).

PGs are cyclic five-carbon structures; the two COX isoforms generating PGs consist of a long narrow channel with a hairpin bend at the end. For COX-2 the substitution of valine for isoleucine creates a wider channel than for COX-1. Selective COX-2 inhibitors have a broader conformation, so that they enter the COX-1

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channel less readily. A further difference is the kinetics of binding of NSAIDs to each isoform. COX-1 inhibition involves hydrogen bonding, is instantaneous and competitively reversible, except for aspirin. COX-2 inhibition may involve covalent binding, is time dependent, and slowly reversible.

Eicosanoid receptors are cell membrane spanning and G-protein coupled; several subdivisions have been classified, corresponding to each COX metabolite, for example DP for PGD_2, EP for PGE_2, FP for $PGF_{2\alpha}$, and IP for PGI_2. The actions of PGE_2 are mediated by a range of receptor subtypes (EP1, EP2, EP3, and EP4) activation of which leads to ongoing intracellular signaling pathways. The EP4 receptor is associated with inflammatory pain and has been implicated in rheumatoid and osteoarthritis (OA). Grapiprant, a selective EP4 receptor antagonist, is under development for use in dogs with OA (Rausch-Derra and Rhodes, 2015).

Following synthesis, PGs must exit the cell; ABC genes provide ABC transporters to consume ATP, facilitating transfer across cell membranes. For example, MRP4 has particular affinity for transporting PGE_2, TxA_2, and $PGF_{2\alpha}$ (Warner and Mitchell, 2003).

Several examples of synergism between proinflammatory PGs and other inflammatory mediators may be cited. First, both histamine (Chapter 19) and bradykinin are primary inflammatory mediators, which stimulate nociceptors (peripheral nerve endings) to increase the discharge in afferent nerves, so that pain is sensed in spinal and brain centers. PGE_2 synergizes with these primary mediators to increase both intensity and duration of the afferent discharge, a phenomenon termed *hyperalgesia* (Ferreira, 1983). In addition, when tissues are damaged, stimuli (e.g., touch) that are not normally painful, become painful, a phenomenon termed *allodynia*; PGs are again implicated (Nolan, 2001). It is by inhibiting PG synthesis that NSAIDs exert their analgesic actions.

Second, histamine and bradykinin increase endothelial intercellular gaps in capillary and small postcapillary venules, to increase loss of plasma into the interstitial space. This alteration to the Starling balance of forces causes exudation of plasma, accounting for one of the classical signs of acute inflammation, edema. PGs do not alter capillary permeability directly but, by dilating small arterioles, they enhance the edema induced by the primary mediators. Inhibition of synthesis of PGs by NSAIDs explains their antiedematous actions.

Third, there is synergy between proinflammatory mediators in the induction of COX-2 protein and in the supply of AA to enhance prostanoid production at inflammatory sites. Hamilton et al. (1999) showed that lipopolysaccharide (LPS)-induced COX-2 induction alone did not greatly increase prostanoid production, but it was markedly increased by bradykinin administration through an increase in the supply of the substrate AA.

COX-2 generates *antiinflammatory* as well as *proinflammatory* PGs; COX-2 is up-regulated not only in the early stage (2 hours) of acute inflammation but also in the resolution phase (around 48 hours). The early peak of COX-2 production is associated with the synthesis of proinflammatory PGE_2 by polymorphonuclear leukocytes, while the later peak is associated with the synthesis of antiinflammatory PGs (Gilroy et al., 1998, 1999). These include 15deoxy$\Delta^{12-14}PGJ_2$ ($15dPGJ_2$) synthesized by mononuclear cells; $15dPGJ_2$ is a ligand for the nuclear receptor peroxisome proliferator-activated receptor-γ (PPAR-γ) and some of its actions may result from activation of PPAR-γ (Kawahito et al., 2000). Inhibition of synthesis of $15dPGJ_2$ with COX-2 selective as well as with nonselective NSAIDs theoretically might lead to a more protracted resolution/ healing phase in acute inflammation (Konturek et al., 2005) although this has not been identified as a clinical problem.

As well as the peripheral/ local roles of PGs, they are involved in pain perception at the spinal level. Spinal nociceptive processing is facilitated by firing of neurons and enhancement of transmitter release from primary spinal sensory afferents. Furthermore, PGE_2 is also an endogenous pyrogen, leading to upward resetting of the temperature regulating center in the anterior hypothalamus.

Proinflammatory PGs and leukotrienes (LTs) are synthesized from AA by the catalytic actions of COX and 5-LO, respectively. ω-3 fatty acids, present in high concentrations in fish oils, include eicosapentanoic acid (EPA) and docosahexanoic acid (DHA). EPA and DHA also serve as substrates for COX and 5-LO. They may thereby suppress the proinflammatory activities of AA both competitively and through the synthesis of products with less inflammatory or even antiinflammatory activity. 12-LO leads to 12-hydroperoxyarachidonic acid (12-HPETE) and 12-hydroxyarachidonic acid (12-HETE) formation, while 15-LO forms lipoxins A and B. The lipoxins possess antiinflammatory properties; together with $15dPGJ_2$ they may subserve roles in the resolution stage of acute inflammation. On the other hand, 5-LO generates the LT family of proinflammatory eicosanoids, such as LTB_4 and the peptidoleukotrienes LTC_4, LTD_4, and LTE_4. LTB_4 is a potent proinflammatory chemoattractant, drawing first neutrophils and later mononuclear cells to inflammatory sites. Peptidoleukotrienes are potent bronchoconstrictors. LTs are mediators of local vascular and cellular changes at sites of nonimmune inflammation and also in immune-mediated inflammatory conditions. Possible roles of LTs in conditions such as reactive airway obstruction in the horse and skin allergies in the dog have been investigated, and the use of 5-LO inhibitors as well as inhibitors of the actions of released LTs as therapeutic agents in immune-based inflammatory diseases has been researched (Marr, 1998).

Table 20.1 Characteristics, actions, and roles of COX-1 and its inhibition

A membrane-bound hemo- and glycoprotein of molecular weight 71 kDa, present in the endoplasmic reticulum
Encoded by a 22-kb gene
Constitutively expressed by cells in a wide range of tissues; involved in "housekeeping" functions
Enzyme concentrations are relatively stable, although small (two- to fourfold) increases occur in response to stimulation by hormones and growth factors
Responsible for generation of TxA_2 and PGs, e.g., PGE_2 and PGI_2 with local actions, such as gastroprotection, renoprotection, and hemostasis
Inhibited by classical NSAIDs which generally are nonselective for COX-1 and COX-2
NSAIDs, which may show some selectivity for inhibition of COX-1 relative to COX-2, include aspirin and ketoprofen.
Aspirin irreversibly inhibits COX-1 by covalent acetylation of amino acid Ser 530. Other NSAIDs inhibit COX-1 reversibly by excluding arachidonic acid from the upper portion of a long, hydrophobic channel

COX, cyclooxygenase; NSAID, nonsteroidal antiinflammatory drug; PG, prostaglandin.

Isoforms of Cyclooxygenase: Characteristics, Locations, and Roles

Cyclooxygenase-1 (COX-1)

COX-1 is a membrane-bound enzyme present in the endoplasmic reticulum (Table 20.1). It first cyclizes AA to form PGG_2 and then adds a 15-hydroperoxy group to convert PGG_2 to PGH_2. COX-1 is expressed constitutively in most tissues. It is involved in "housekeeping" functions, including blood clotting, regulation of vascular homeostasis, renoprotection, gastroprotection, and coordination of the actions of circulating hormones.

Cyclooxygenase-2 (COX-2)

COX-2 is both an inducible and constitutive isoform (Table 20.2) (Wooten et al., 2008). COX-2 synthesis is stimulated by proinflammatory cytokines, growth factors, lipopolysaccharide (LPS), and mitogens. The cloning, expression, and selective inhibition of canine COX-1 and COX-2 have been reported (Gierse et al., 2002). While most data support COX-2 as the isoform which generates pro- and antiinflammatory PGs at sites of inflammation, some data indicate a role for COX-1 as well (Smith et al., 1998; Bertolini et al., 2001).

Table 20.2 Characteristics, actions, and roles of COX-2 and its inhibition

Molecular weight of 70 kD and having 60% homology with COX-1 at the amino acid level
Has similar active sites to COX-1 for binding AA and NSAIDs, although the active site of COX-2 is larger and more flexible than that of COX-1 and can accept a wider range of structures as substrates
Encoded by an 8.3-kb gene
One of a family of primary response genes induced during inflammation and cell growth
Unlike COX-1 possesses a TATA box and binding sites for transcription factors, e.g., NFκB and a cyclic AMP response binding element in the promoter region of the immediate-early gene
A better competitor than COX-1 for AA released within the cell
Expression increased on exposure to lipopolysaccharide, cytokines (e.g., IL-1, TNF-α) immune and inflammatory stimuli
Present constitutively in monocytes, macrophages, pyloric and duodenal mucosa, endothelial cells, brain, dorsal horn cells of spinal cord, kidney, ovary, uterus, and ciliary body in the eye
Produces proinflammatory PGs (e.g., PGE_2) in the early stages of the acute inflammatory response and antiinflammatory PGs (e.g., $15dPGJ_2$) in the resolution phase
Down-regulated at the mRNA level by corticosteroids.
Exerts significant roles in certain cancers, Alzheimer's disease, and arthritides, and activation inhibits apoptosis (NSAIDs induce apoptosis)
Aspirin preferentially inhibits COX-1 (acetylating Ser 530), although higher concentrations also acetylate irreversibly Ser 516 on COX-2. Other NSAIDs compete reversibly with AA, the substrate for COX-1 and COX-2 for the active sites on the enzymes
Specific and selective inhibitors of COX-2, the COXIBs, are antiinflammatory and analgesic and have better gastrointestinal tolerance than many nonselective COX inhibitors

AA, arachidonic acid; COX, cyclooxygenase; NSAID, nonsteroidal antiinflammatory drug; PG, prostaglandin.

History of NSAIDs

The antecedents of the modern range of NSAIDs were extracts of various plants, in therapeutic use for more than 3,500 years, and comprising particularly the leaves and bark of the willow tree. These contain salicyl alcohol in free form or as glycosides such as salicin. The antiinflammatory properties of willow extracts were described by Dioscorides in his pharmacopoeia in the first century AD, as follows: "the leaves being beaten small and dranke with a little pepper and wine doe help such as are troubled with the Iliaca Passio (colic) … the concoction of ye leaves and barke is an excellent fomentation for ye gout." The Rev. Edward Stone described, in 1795, the antipyretic properties (cure of agues) of the willow noting, "as this tree delights in a moist or wet soil, where agues (fever) chiefly abounds the general maxim that many natural remedies carry their cures along with them or that remedies lie not far from their causes was so very apposite to this particular case that I could not help applying it." The "like cures like" concept was fashionable at that time, and persists in the nonscience of homeopathy to this day.

Discovery of salicyl alcohol/ salicylic acid as the active principles of plant extracts led to the use of sodium salicylate as the first synthetic NSAID in 1875 and then to the introduction of the acetyl ester of salicylic acid (aspirin) in 1898. By the sixth decade of the 20th century, aspirin, cinchophen, phenylbutazone, dipyrone, and isopyrin were, for several years, the commonly used NSAIDs in veterinary therapy. In the 1970s, flunixin was introduced and subsequently many novel NSAIDs have received marketing authorizations. Over the last 20 years in particular, there have been intensive searches for novel agents within the NSAID class, stimulated by several factors.

First has been the recognition that animals feel and suffer pain in a manner similar to the human animal. The welfare benefit of controlling animal pain is widely recognized. Second, it has been recognized that available drugs of the NSAID class, while commonly very efficacious, do not consistently in all animals or in all circumstances provide adequate levels of analgesia, especially when pain is severe. Third, the need to improve safety margins, notably in relation to gastrointestinal (GI) tolerance of NSAIDs, but also to ensure renal safety and to avoid uncontrolled hemorrhage, has been acknowledged. Most NSAIDs are clinically safe when used at recommended dose rates. However, there are several qualifications: there are equine examples (see Sections Additional actions of NSAIDs: gastrointestinal toxicity; renotoxicity; and hepatotoxicity) of narrow margins between clinically recommended NSAID doses and doses causing significant toxicity; side effects may be related to age and physiological/ pathological status (e.g., conditions and circumstances involving hypotension and hypovolemia predispose towards renotoxicity); NSAID toxicity may be idiosyncratic, occurring rarely but unpredictably, even with manufacturers' recommended dose rates. Therefore, safety concerns on older drugs have driven the search for novel agents.

Classification of NSAIDs, Chemical Structures, and Physicochemical Properties

Classifications based on chemical structure are of limited value because, despite minor differences between subgroups, all older NSAIDs have similar pharmacological actions (analgesic, antiinflammatory, and antipyretic), toxicity profiles, and clinical uses. Moreover, physicochemical properties are generally similar; almost all are weak organic acids (pKa in the order of 3.5–6.0) and of moderate to high lipid solubility (Veterinary Medicine Expert Committee on Drug Information, 2004).

The older (classical) NSAIDs are divided on the basis of chemical structure into two main groups: carboxylic acids and enolic acids, further divided into subgroups on the basis of chemical structure (Table 20.3, Figure 20.1). NSAIDs of the 2-arylpropionate group contain a single center of asymmetry; they are therefore chiral compounds. They are produced commercially as racemic (50 : 50) mixtures of the two optical enantiomers (R[−] and S[+]) and thus comprise a mixture of two drugs with differing pharmacokinetic and pharmacodynamic profiles.

Table 20.3 Chemical classification of classical NSAIDs

Carboxylic acids (R-COOH)	Enolic acids (R-COH)
Salicylates	**Oxicams**
Sodium salicylate[b]	*Meloxicam*[a]
Acetylsalicylic acid[a]	Piroxicam
Indoleacetic acids	Tenoxicam
Etodolac[a]	**Pyrazolones**
Indolines	*Phenylbutazone*[a]
Indomethacin	Oxyphenbutazone[c]
Thiopheneacetic acids	*Isopyrin (ramifenazone)*[a]
Diclofenac	*Dipyrone*[a]
Eltenac[a]	
2-Arylpropionic acids	
Carprofen[a]	
Ketoprofen[a]	
Vedaprofen[a]	
Ibuprofen	
Anthranilic acids	
Flunixin[a]	
Meclofenamic acid[a]	
Tolfenamic acid[a]	
Quinolines	
Cinchophen[a]	

[a] Drugs currently or previously licensed for veterinary use in some countries.

[b] Also a metabolite of acetylsalicylic acid.

[c] Also an active metabolite of phenylbutazone.

Novel SAID Classes

COX-2 inhibitors: The COXIBs are preferential or selective inhibitors of COX-2. They have differing structures to classical NSAIDs (Figure 20.1). Most are sulphones or sulphonamides, although robenacoxib is exceptional, being a carboxylic acid. The sulphones and sulphonamides are lipophilic compounds. Their relatively bulky structure limits COX-1 inhibition by steric hindrance. They have provided a significant advance in pain therapy (Wilson et al., 2004). The introduction of firocoxib into canine medicine for the therapy of canine OA comprised the first COX-2 selective drug for veterinary use (McCann et al., 2004), and others, including cimicoxib, deracoxib, mavacoxib, parecoxib, and robenacoxib, have followed.

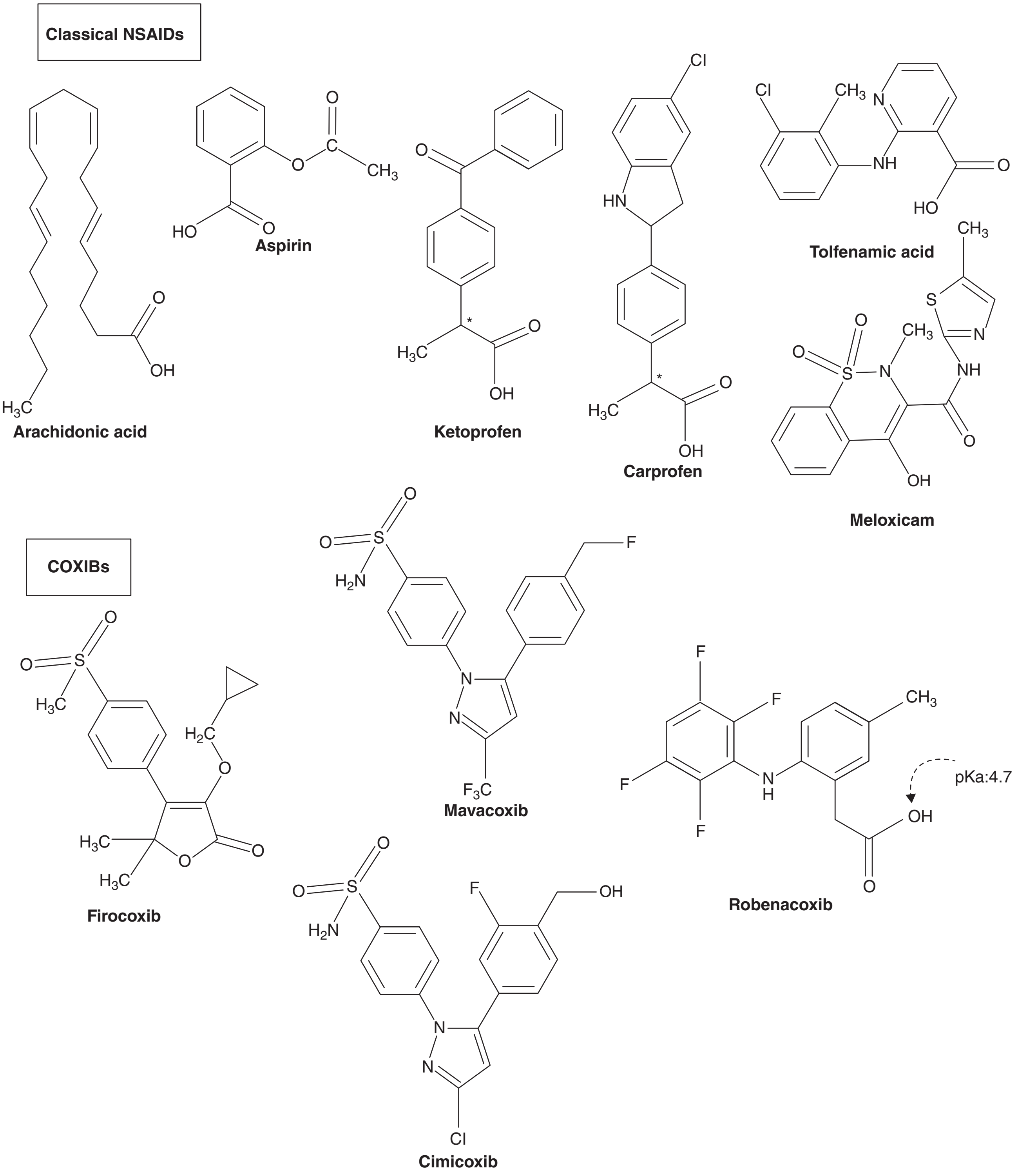

Figure 20.1 Structures of arachidonic acid and classical NSAIDs (aspirin, ketoprofen, carprofen, tolfenamic acid, and meloxicam) and COXIBs [firocoxib (methylsulfone), mavacoxib / cimicoxib (sulphonamides) and robenacoxib (carboxylic acid)]. * site of asymmetric carbon atom.

CINODS: COX-inhibiting nitric oxide donors (CINODs) are nitrosoesters of the older nonselective COX inhibitors (e.g., aspirin, phenylbutazone). The ester linkage is hydrolyzed in vivo to yield parent NSAID and the vasodilator nitric oxide (NO). The latter may enhance potency and increase gastric tolerance (Wallace et al., 2004). No drug of this class has been introduced into veterinary therapeutics.

Dual COX/5-LO inhibitors: The dual inhibitor tepoxalin was introduced into canine medicine (Argentieri et al., 1994) but drugs of this class have found only limited use in veterinary medicine.

Pharmacology of NSAIDs

NSAID Pharmacokinetics

Absorption

As lipid-soluble weak organic acids, classical NSAIDs are generally well absorbed when administered orally (Table 20.4), although rate and extent of absorption vary with species, gastric pH, dosing in relation to feeding, and gastrointestinal motility. In monogastric species, absorption from the stomach is favored by the Henderson–Hasselbalch ion trapping mechanism, which maintains a diffusion gradient for undissociated acid molecules between acidic gastric juice and plasma of pH 7.4. In ruminants, initial absorption from the four-compartment stomach and subsequent intestinal absorption creates the basis for a biphasic absorption pattern. In young ruminants, operation of the esophageal groove reflex is another mechanism accounting for double peaks in plasma concentration (Marriner and Bogan, 1979). Despite dilution in a large volume of rumen liquor (approximately 120 liters in an adult cow), bioavailability of phenylbutazone and meclofenamate was shown to be 50–60% in cattle (Lees et al., 1988a; Marriner and Bogan, 1979).

Table 20.4 General pharmacokinetic properties of NSAIDs

Generally good bioavailability in monogastric species after oral dosing. Absorption may be (a) delayed by binding to digesta (e.g., in horses and ruminants) (b) enhanced or reduced in the presence of food
Good bioavailability after parenteral (IM, SC) dosing
Penetrate blood–brain barrier to act centrally
High degree of plasma protein binding of all drugs (except salicylate) in all species limits passage from plasma into interstitial and transcellular fluids but *facilitates* passage into inflammatory exudate
Glomerular ultrafiltration and the renal excretion of parent drug markedly limited by high degree of plasma protein binding
Low volume of central compartment and low volume of distribution (with some exceptions, e.g., most COXIBs)
Elimination predominantly by metabolism in liver, usually to inactive compounds, but some metabolites are active, e.g., Phenylbutazone → oxyphenbutazone Aspirin → salicylate
For most drugs marked species (and possibly breed) differences in clearance and elimination half-life
Reduced clearance, increased half-life in neonates
Biliary secretion and enterohepatic recycling for some drugs

COXIB, cyclooxygenase inhibitor.

There are exceptions to the high bioavailability of NSAIDs after oral dosing. Bioavailability in horses was <5% for an oil-based formulation of RS-ketoprofen. However, when the pure drug substance was administered, bioavailability was of the order of 50% (Landoni and Lees, 1995). Another factor affecting absorption rate, of significance in the horse, is binding to hay (shown in vitro) and to digesta (demonstrated in vivo) (Lees et al., 1988b). Binding delayed the absorption of phenylbutazone and flunixin, accounting for double peaks in plasma concentration–time curves (Maitho et al., 1986; Welsh et al., 1992). In monogastric species, administration of NSAIDs with food is a common practice, as it may lessen irritant effects on the GI. As in horses, this practice is likely to delay absorption but to reduce absorption only moderately or not at all. However, the bioavailability of mavacoxib was higher in fed (87%) than in fasted (46%) dogs, while the converse was true for robenacoxib in both the dog (62 and 84%, respectively) and cat (10 and 49%, respectively) (Cox et al., 2010; Jung et al., 2009; King et al., 2013). As well as feed, other factors, including product formulation, may influence the rate and/or extent of absorption of NSAIDs. For example, aspirin is available in plain, water-soluble, film-coated, buffered, enteric-coated, and time-release formulations, with differing disintegration and dissolution rates.

Distribution

Most NSAIDs are highly bound to plasma protein (95–99% or greater). This limits passage from plasma into interstitial fluid. The volumes of the central compartment and distribution are generally low and values of 0.1–0.3 l/kg or less are common. There are exceptions, however, and moderate to high volumes of distribution have been reported for flunixin in cattle (but not in other species); for tolfenamic acid in the dog, calf, and pig; and for all sulphones and sulphonamide COXIBs (but not for the carboxylic acid derivative robenacoxib) in the dog. Contributing to these high distribution volumes for COXIBs is enterohepatic recirculation or a high level of extravascular accumulation/ tissue binding.

A potential therapeutic advantage of the high degree of plasma protein binding is accumulation of NSAIDs in inflammatory exudate. Since exudate is rich in albumin, which has leaked from the circulation, the binding of NSAIDs to albumin accounts for ready penetration into and persistence at sites of acute inflammation

Table 20.5 Examples of penetration into acute inflammatory exudate and transudate relative to plasma

Administered drug	Species	Dose (mg/kg)	AUC (µg·h/ml)		
			Plasma	Exudate	Transudate
RS-carprofen[b 1]	Horse	4.0	558 (R) 138 (S)	451 (R) 133 (S)	336 (R) 93 (S)
Flunixin[a 2]	Calf	2.2	11.8	27.6	7.0
Flunixin[a 3]	Horse	1.1	19.3	36.0	12.1
S-ketoprofen[a 4]	Horse	1.1	2.7	32.6	3.4
S-ketoprofen[a 5]	Calf	1.5	13.2	26.1	20.9
Phenylbutazone[a 6]	Horse	4.4	156	128	49
Phenylbutazone[a 7]	Donkey	4.4	19.2	8.0	5.0
Phenylbutazone[b8]	Calf	4.4	3604	1117	766

[a] Short plasma elimination half-life;
[b] longer half-life.
Data from [1]Armstrong et al. (1999a); [2]Landoni et al. (1995c); [4]Landoni and Lees (1996); [5]Landoni and Lees (1995); [6]Lees et al. (1986); [7]Cheng et al. (1996); [8]Arifah et al. (2002).

(Table 20.5). Exudate concentrations of NSAIDs often exceed those in plasma when drug body clearance is high and terminal half-life is short. Accumulation in exudate is a likely explanation for: (i) the maintained effectiveness of NSAIDs when plasma concentrations have decreased to low levels and (ii) why those NSAIDs with short elimination half-lives, for example flunixin, ketoprofen, vedaprofen, tolfenamic acid, and robenacoxib, may be effective with once-daily dosing. This longer duration of effect than predicted from plasma concentration–time profile is associated with the negative hysteresis described in in vivo studies, which indicate that maximal inhibition of PGE_2 synthesis in exudate occurs some time after peak drug concentrations in plasma (Giraudel et al., 2005b; Pelligand et al., 2012, 2014). The latter group reported elimination half-lives from feline exudate for S-ketoprofen and robenacoxib of 26 and 41 hours, respectively; corresponding values for blood were 1.62 and 1.13 hours after subcutaneous administration. Exudate PGE_2 concentration was reduced for 24–36 hours. In clinical OA dogs, the synovial fluid concentration of robenacoxib exceeded the IC_{50} for COX-2 for 16 hours; the corresponding time for healthy beagles was 10 hours (Silber et al., 2010).

For the 2-arylpropionate NSAIDs, enantioselectivity of distribution into exudate (Table 20.5) and synovial fluid (Armstrong et al., 1999a) has been demonstrated. This impinges on efficacy, as the two enantiomers exhibit pharmacodynamic differences as well (see Section NSAID pharmacodynamics: Inhibition of COX and 5-LO).

For those COXIBs based on sulphonamide or sulphone structures, the molecules are nonionized at physiological pH and volumes of distribution are high, whereas robenacoxib, a carboxylic acid, resembles most nonselective NSAIDs in having a low distribution volume.

A possible consequence of NSAID plasma protein binding is competition with other protein-bound drugs; theoretically this can lead to acute toxicity of either drug as the free concentration is increased. Nephrotic animals have reduced plasma protein concentrations and some authors have suggested that this may predispose to NSAID toxicity. However, in both of these circumstances, this will rarely be clinically significant, as the free concentration quickly falls, being subject to redistribution, metabolism, and excretion (Toutain and Bousquet-Melou, 2002).

The penetration of NSAIDs into milk in the absence of mammary gland infection is poor, milk concentrations being of the order of 1% or less of plasma total concentration. This results from the high degree of binding to plasma protein. Distribution into milk is also limited by the Henderson–Hasselbalch mechanism, as milk pH is less than that of plasma. In mastitis, however, penetration is likely to be increased from these low levels.

Excretion and Metabolism (Elimination)

As classical NSAIDs are weak organic acids, elimination in urine might be expected to vary with urine pH. In principle, ion trapping of the poorly lipid-soluble ionized moiety in alkaline urine will favor excretion in herbivores (horses and ruminants) but not in omnivores and carnivores (dogs and cats), as their urine is generally acidic. However, of over-riding importance for urinary excretion in all species is the high degree of plasma protein binding, which limits passage into glomerular ultrafiltrate to a small proportion of the total drug concentration in plasma. Hence, for most NSAIDs only small fractions of the administered dose are excreted in unchanged form in urine, irrespective of species and urine pH. This is illustrated by phenylbutazone excretion in the alkaline urine of horses; as a percentage of administered dose, the 24-hour excretion was 1.9 (Lees et al., 1987b). There is evidence for biliary secretion of some NSAIDs (e.g., carprofen) creating the basis for possible enterohepatic recirculation (Priymenko et al., 1993). Mavacoxib is also secreted in bile and its prolonged elimination half-life is due, in part, to repeated reabsorption of secreted drug but also to its high distribution volume (Lees et al., 2015).

Most NSAIDs are eliminated primarily by hepatic metabolism to less active (or inactive) phase 1 metabolites, which undergo conjugation phase 2 reactions to more polar, readily excreted conjugates. However, some drugs, such as aspirin and phenylbutazone, are converted to active metabolites, salicylate and oxyphenbutazone, respectively (Lees et al., 1987a,b). The deacetylation of aspirin is, in part, a spontaneous reaction, so that aspirin has a half-life of only several minutes in plasma. Therefore, salicylate accounts for most of the analgesic and antiinflammatory properties of administered aspirin, although aspirin itself accounts for the platelet-based antithrombotic actions. This is because aspirin blocks platelet COX covalently and irreversibly and therefore for the lifespan of the platelet. This permanent inhibition of platelet COX occurs after exposure for only a limited period, so that the short half-life of aspirin (approximately 9 minutes) in the horse does not limit platelet COX-1 inhibition for several days (Lees et al., 1987a).

Examples of elimination half-life are presented in Table 20.6. Interpretation of these values must take account of the fact that half-life is a hybrid variable, controlled by body clearance and volume of distribution.

Species differences in some pharmacokinetic variables are the rule rather than the exception for NSAIDs. For example, while plasma protein binding is almost invariably very high and volumes of distribution are low for classical NSAIDs in most species, clearance and terminal half-life vary markedly. The example of phenylbutazone is presented in Table 20.7. Aspirin, like phenylbutazone, provides a useful example of the impact of elimination on dosing intervals. The terminal half-life of its metabolite salicylate ranges from 32 minutes in cattle to 22–45 hours in the cat, in which species elimination is zero order (dose-dependent pharmacokinetics).

Table 20.7 Species differences in phenylbutazone pharmacokinetics

Species	Elimination half-life (h)	Clearance (ml/h/kg)
Man	72–96	—
Cow	42–65	1.24–2.90
Sheep	18	—
Goat	16	13.0
Camel	13	4.9–10.0
Horse	4–6	16.3–26.0
Dog	4–6	—
Rat	2.8–5.4	35–86
Donkey	1–2	170

The pharmacokinetics of the 2-arylpropionate subgroup of NSAIDs (carprofen, ketoprofen, and vedaprofen) is influenced by the possession of a single chiral center. There are therefore two mirror-image enantiomeric forms, R and S. The licensed products are the racemic (50 : 50) mixtures. Etodolac, though not a 2-arylpropionate, also possesses a single chiral center. The importance of NSAID chirality derives from the fact that the body is a chiral environment, whose cell membranes, macromolecules, and enzymes are based on D-monosaccharides and L-amino acids. Therefore, while NSAID enantiomers have virtually identical physicochemical properties, they do *not* have the same pharmacological properties in the body's chiral environment, as manifested by both pharmacokinetic and pharmacodynamic differences. Pharmacokinetic data based on "the total drug" concept must be viewed with alarm; such data comprises "highly sophisticated scientific nonsense" (Ariens, 1985) because racemic mixtures are simply combinations, in equal proportions, of two distinct drugs. Pharmacokinetic differences are reflected in clearance, elimination half-life, and plasma area under curve (AUC) ratios for the two enantiomers. As illustrated by

Table 20.6 Terminal half-life of NSAIDs (intravenous administration unless stated)

Species	Salicylate	Flunixin	Meloxicam	Carprofen S(+) R(−)	Ketoprofen S(+)R(−)	Naproxen	Tolfenamic Acid
Horse	1.0–3.0	1.6–2.1	3	16, 21	1.0, 0.7	5	7.3
Cow/calf	0.5, 3.7 (PO)	8	13	37, 50	0.4, 0.4	—	11.3
Pig	5.9	—	4	—	—	5	3.1
Dog	8.6	3.7	12–36	7, 8	3.5[c]	35–74[b]	5.3
Cat	22–45[a]	—	37	15, 20	1.5, 0.6	—	10.8
Monkey	—	—	—	—	—	1.9	—
Man	3.0 (PO)	—	20–50	12[c]	—	14	—

[a] Dose-dependent pharmacokinetics.

[b] Breed-dependent pharmacokinetics (lower value refers to beagles, higher value to mongrels).

[c] Values quoted in the literature for total drug (combined R and S enantiomers) a concept that is flawed. For the COXIBs see Table **20.**12.

Table 20.8 Species variation in stereoselective pharmacokinetics of carprofen enantiomers[a]

Species	Dose of Rac-carprofen (mg/kg)	Administration route (and dosing duration)	AUC (% of total)	
			R (−)	S (+)
Dog (beagle)[1]	4.0	Oral	64	36
Dog (various breeds)[2]	2.0	Oral (day 1)	52	48
		Oral (day 7)	52	48
		Oral (day 28)	57	43
Cat[3]	0.7	Intravenous	69	31
	0.7	Subcutaneous	67	33
	4.0	Intravenous	70	30
	4.0	Subcutaneous	72	28
Calf[4]	0.7	Intravenous	58	42
Horse[5,6]	0.7	Intravenous	80–84	16–20
	4.0	Intravenous	80	20
Sheep[7]	4.0	Intravenous	74	26

[a] Single dose unless stated.
[1] McKellar et al., 1994.
[2] Lipscomb et al., 2002.
[3] Taylor et al., 1996.
[4] Delatour et al., 1996.
[5] Lees et al., 2002.
[6] Armstrong et al., 1999a.
[7] Cheng et al., 2003.

carprofen, AUC ratios vary between species and may be as high as 4 : 1, despite the 1 : 1 ratio in the administered products (Tables 20.5 and 20.8).

Differences in pharmacokinetics for each enantiomer of chiral NSAIDs may arise in two ways. First, there are enantiomer differences in elimination, arising from differing rates of hepatic metabolism (Soraci et al., 1995). Second, for some drugs chiral inversion occurs in vivo. This, almost invariably, is unidirectional, comprising R to S inversion. Species (and breed) differences in the extent of chiral inversion of ketoprofen have been investigated (Table 20.9). There is also inevitable interanimal variation in the pharmacokinetics of NSAIDs. Table 20.10 presents data for firocoxib in beagles.

As well as interanimal and interbreed differences, a report on celecoxib in a colony of 245 beagle dogs revealed a *within*-breed difference; two subpopulations were identified, an extensive metabolizer (EM) phenotype for which mean half-life was 1.72 hours and clearance was 18.2 ml/kg/min, and a poor metabolizer (PM) phenotype for which corresponding values were 5.18 h and 7.15 ml/kg/min (Paulson et al., 1999). This example points to the possibility of significant genetic differences in clearance and terminal half-life of other NSAIDs. In fact, similar within-breed (beagle) pharmacokinetic differences (EM and PM phenotypes) have been described for cimicoxib (Jeunesse et al., 2013). Respective clearances were 0.31 and 0.11 l/h/kg. In a lameness model, duration of action was greater in the PM dogs. These authors also administered cimicoxib to dogs of four breeds; half-lives were of the same order of magnitude as those of the EM beagles.

In addition to species, inter- and intrabreed, and interanimal differences in NSAID pharmacokinetics,

Table 20.9 Species differences in extent of chiral inversion of R(−)- to S(+)-ketoprofen

Species	Inversion (% of administered dose)
Horse	48.8
Calf	31.7
Cat	22.4
Goat	15.0
Sheep (female, Dorset Cross)	13.8
Sheep (male, Corriedale)	5.9
Man	8.9

Table 20.10 Interanimal variation in firocoxib pharmacokinetics in the beagle dog[a]

Parameter	Mean	Range
C_{max} (μg/ml)	1.01	0.51–1.37
T_{max} (h)	2.63	0.79–4.45
AUC (μg/ml·h)	11.00	8.55–14.27
$T_{1/2el}$ (h)[b]	6.31	3.31–9.99

[a] Eight young beagles, both sexes: 5 mg/kg single dose administered orally (McCann et al., 2004).
[b] T1/2 values reported for other species are: 9–12 h (cat) and 30–40 h (horse).

there are inevitable intraanimal differences, arising from pathological or physiological state. Lascelles et al. (1998) reported, in dogs undergoing anesthesia, greater values of C_{max} (20.6 versus 11.0 μg/ml) and AUC (175 versus 115 μg/ml·h) when carprofen was administered postoperatively compared to preoperatively. In adult cows with an *E. coli* endotoxin-induced mastitis, values of AUC and clearance were 507 mg/l·h and 1.4 ml/kg/h for carprofen (Lohuis et al., 1991) whilst values for healthy cows were 294 mg/l·h and 2.4 ml/kg/h. Age may affect the pharmacokinetics of NSAIDs. Clearance is likely to be slower and half-life longer in neonates, as established for phenylbutazone in goats (Eltom et al., 1993) and as suggested for carprofen in young calves (Lees et al., 1996; Delatour et al., 1996) and for phenylbutazone in older horses (Lees et al., 1985). The effect of an endurance exercise on phenylbutazone pharmacokinetics in the horse was investigated by Authie et al. (2010) in relation to medication control; hepatic clearance was decreased, as also was volume of distribution, so that terminal half-life was not changed.

In the dog the clearance of COXIBs differs between the extremes of robenacoxib and mavacoxib (Table 20.11); 13.5 ml/min/kg (robenacoxib) and 0.045 ml/min/kg (mavacoxib) and respective half-lives were 0.63 hours and 17 days in laboratory (beagle or mongrel) dogs. In the cat the elimination half-life of robenacoxib was 0.78 to 1.49 hours and was independent of administration route – intravenous, oral, or subcutaneous (King et al., 2013; Pelligand et al., 2012). Mavacoxib biotransformation and renal excretion in the dog are very limited; elimination is predominantly by biliary secretion and excretion in feces, and enterohepatic recirculation contributes to the slow clearance and long terminal half-life. Lees et al. (2015) calculated that the maximum amount excreted daily in bile was less than 10% of the monthly dose. Based on dose normalized plasma AUC, the pharmacokinetics of mavacoxib administered orally did not differ between mongrel and beagle dogs and was proportional to dose over the range 2–25 mg/kg (Cox et al., 2010).

However, in population pharmacokinetic studies, in mainly elderly large breed dogs with OA, the mean elimination half-life of mavacoxib was longer (44 days) than the half-life of 17 days in laboratory dogs and in approximately 5% of OA dogs $T_{1/2}$ exceeded 80 days. This led to reduction in the dose predicted from laboratory dogs of 4 mg/kg to 2 mg/kg, with a dosing interval of 14 days between first and second doses and 28 days between subsequent doses and with a duration of therapy limited to 6 months (Cox et al., 2011). These authors reported body weight as the primary factor predicting clearance and distribution volume (both scaled by bioavailability) and with smaller effects of age and breed. In a clinical population of OA dogs, it was shown that apparent clearance and volume of distribution of robenacoxib were likewise proportional to body weight (Fink et al., 2013). Clearly, there is a need to conduct population pharmacokinetic studies in clinical subjects on other NSAIDs.

Parecoxib is an inactive prodrug, with a short half-life (0.42 hours) after intramuscular injection in the cat; it is converted in vivo to the active metabolite valdecoxib ($T_{1/2}$ = 8.52 hours; Kim et al., 2014).

Baert and De Backer (2003) provided valuable comparative pharmacokinetic data for sodium salicylate, flunixin, and meloxicam in five bird species, chicken, ostrich, duck, turkey and pigeon; species differences were marked.

Drug Residues

Regulatory authorities require that meat and milk withholding periods be established for NSAIDs licensed for use in food-producing species, including the horse. These are set on the basis of each drug's

Table 20.11 Comparative pharmacokinetics of five COXIBs in Beagle or mongrel dogs[a]

Variable (units)	Route	Mava coxib	Robenacoxib	Firocoxib	Deracoxib	Cimicoxib[c]	Cimicoxib[d]
Cl (ml/min/kg)[b]	IV	0.045	13.5	7.7	5	5.2	1.8
$t^1/_2$ (h or days)	IV	17 days	0.63 h	5.9 h	3 h	2.72 h	5.63 h
Vd_{ss} (l/kg)	IV	1.64	0.24	2.9	1.5	1.12	0.89
Plasma protein binding (%)	IV	>98	>98	–	>90	–	–
T_{max} (h) (fed dogs)	Oral	17.4	0.25	–	2.0	–	–
T_{max} (h) (fasted dogs)	Oral	67.4	0.5	1.0	–	–	–
F (%) (fed dogs)	Oral	87	62	–	>90	–	–
F % (fasted dogs)	Oral	46	84	101	–	–	–

[a] Data from Cox et al. (2010); McCann et al. (2004); Jung et al. (2009); Jeunesse et al. (2013); Deracoxib commercial literature.

[b] Clearance values can be compared with the value of 5.8 ml/kg/min defined by Toutain and Bousquet-Melou (2004) as a "low" value for dogs weighing 10 to 20 kg. This value emphasizes the particularly low clearance of mavacoxib.

[c] Extensive metabolizes.

[d] Poor metabolizes.

Cl, body clearance; $t^1/_2$ elimination half-life; $Vd_{ss,}$ volume of distribution at steady state; T_{max}, time of maximum concentration; F, bioavailability.

pharmacokinetic, metabolism, tissue depletion, and toxicological profiles, together with an appropriate safety margin. The relevant considerations for phenylbutazone have been reviewed (Lees and Toutain, 2013). This area is reviewed in Chapter 61 of this text.

NSAID Pharmacodynamics

Inhibition of COX and 5-LO

In addition to pharmacokinetic differences, a second basis for the variation in clinical response to NSAIDs between species, breeds, and individual animals is differences in their pharmacodynamics. This has been studied at several body levels: molecular, cellular, tissue, and whole animal, and can be considered in terms of the action–effect–response relationship (Table 20.12). The principal *action* is inhibition of COX, the enzyme with a pivotal position in the AA cascade; NSAIDs occupy the hydrophobic channel of COX, preventing AA access to its active site. This leads to the *effect* of inhibition of synthesis of proinflammatory mediators, including PGE_2 and PGI_2. The action and effect have been demonstrated with clinically achieved concentrations of many NSAIDs in several species (Lees and Higgins, 1985; Landoni and Lees, 1995, 1996; Landoni et al., 1995a,b,c; Cheng et al., 2003; Jones et al., 2002). The *responses* are the analgesic, antipyretic, antiinflammatory, antithrombotic, and antiendotoxemic properties of NSAIDs (Lees et al., 1986; Welsh and Nolan, 1994; Welsh et al., 1997; Nolan, 2001; Giraudel et al., 2005b). Current evidence suggests that a high level (80% or higher) of inhibition of PGs may be required to achieve good clinical responses.

Table 20.12 Principal action–effect–response relationships of NSAIDs

Actions	Inhibition of COX-1, COX-2, and, *for some drugs*, 5-LO
Effects	*COX inhibition*: reduced synthesis of eicosanoids with : proaggregatory and vasoconstrictor properties (TxA_2); antiaggregatory and vasodilator properties (PGI_2); proinflammatory properties (PGE_2, PGD_2, PGI_2); and antiinflammatory properties ($15\text{deoxy}\Delta^{12-14}PGJ_2$) *5-LO inhibition:* for dual inhibiting NSAIDs only, decreased synthesis of proinflammatory leukotrienes (LTB_4, LTC_4, LTD_4, LTE_4)
Responses	Reduction of raised body temperature (antipyretic), suppression of pain (analgesic/antihyperalgesic), decreased swelling (antiinflammatory) and *possibly* reduced rate of recovery in the resolution phase of acute inflammation – not clinically significant as far as known

COX, cyclooxygenase; LO, lipoxygenases; PG, prostaglandin.

Vane (1971) discovered that the principal mechanism of action of NSAIDs was inhibition of COX. A further advance occurred with the discovery of two COX isoforms, COX-1 and COX-2 (Kujubu et al., 1991; Xie et al., 1991). It was immediately recognized that most classical NSAIDs inhibit both isoforms, COX-1 inhibition producing toxic effects and COX-2 inhibition providing therapeutic effects. COX-1 was classified as a constitutive enzyme with physiological/ housekeeping functions, including gastro- and renoprotection and blood clotting leading to hemostasis. COX-2 was initially regarded solely as an inducible enzyme, up-regulated at sites of inflammation and responsible for producing proinflammatory mediators. Since 1991, much effort has been directed to identifying the following: which tissues express the two isoforms constitutively; the physiological and pathological roles of each isoform; the role of COX-2 induction at sites of inflammation and centrally; and the nature, incidence, and severity of side effects of drugs that inhibit one or both isoforms.

The overall roles of COX isoforms and the actions of NSAIDs, as perceived in 1991, have been modified as follows:

1) Warner and Mitchell (2003) have concluded that there are only two genes for COX enzymes, COX-1 and COX-2 and not a third, COX-3, as earlier postulated.
2) It is now recognized that COX-2 is a constitutive enzyme, present in stomach, brain, spinal cord, kidney, ovary, uterus, and ciliary body. Therefore, it has been postulated that complete inhibition of COX-2, especially over long periods, might be associated with such side effects as abortion, fetal abnormalities, delayed bone healing, delayed healing of soft tissue (including delayed ulcer healing), renotoxicity, and "adverse cardiovascular events." However, the now extensive clinical use over prolonged periods of COXIBs has been associated with a generally good safety profile. Nevertheless, COXIBs are not free of adverse effects on the GI and there remains controversy over the cardiovascular events associated with their clinical use, especially in human medicine when administered over prolonged periods. COX-2 is both constitutive and inducible in endothelial cells and its selective inhibition might disturb the endothelial PGI_2 (an antiaggregatory and vasodilator PG synthesized via COX-1 and COX-2) platelet TxA_2 (a proaggregatory and vasoconstrictor eicosanoid synthesized by COX-1) balance in the direction of platelet aggregation and vasoconstriction. The cardiovascular events in humans, which have been the subject of much public discussion, might reflect this imbalance. However, some reports have suggested an increased

cardiovascular risk in humans receiving long-term therapy with nonselective as well as COX-2 selective drugs. Epidemiological studies in animals reviewing such risks have not been reported.

3) Some reports indicate that COX-1 may contribute to the synthesis of proinflammatory PGs. Therefore, as well as COX-2 inhibition, inhibition of COX-1, as provided by the older nonselective NSAIDs, might be required for optimal efficacy. However, this concept is controversial and most experimental and clinical data suggest that selective COX-2 inhibitors are as efficacious as nonselective NSAIDs.
4) The introduction of a novel class of NSAIDs, the dual inhibitors, including tepoxalin, which inhibit two enzymes that use AA as a substrate, COX and 5-LO, has failed to make a major impact in veterinary medicine.

Efficacy, Potency, and Sensitivity of Inhibitory Actions of NSAIDs on COX Isoforms

As discussed in Chapter 4, the pharmacodynamic properties of any drug that define and quantify its action on a given tissue, organ, or enzyme are efficacy, potency, and sensitivity. Efficacy (I_{max}/E_{max}) is the maximal response a drug is capable of producing. It is important to the clinician because it defines, for example for a NSAID, the level of pain relief that the drug can provide. Potency is the drug concentration or drug dose producing a given level of response. It is usually determined as the concentration or dose producing 50% of maximal response (EC_{50} or ED_{50}) or, for drugs such as NSAIDs acting to inhibit an enzyme, potency is usually expressed as IC_{50}, although IC_{80} can be more useful. Potency is of less relevance than efficacy to the clinician, but it is critical to pharmaceutical companies when selecting a recommended dose rate for clinical use. NSAID concentration–response data are described by the sigmoidal E_{max} (Hill) relationship (Chapter 4) and the steepness of this relationship (N) determines sensitivity. For NSAIDs, slope may be shallow (less than 1) or steep (10 or greater) and in the latter circumstance the concentration–effect relationship becomes almost quantal (all-or-none).

In vitro assays have been used to determine I_{max}, IC_{50}, IC_{80}, IC_{95}, etc., and N of NSAIDs for each COX isoform. Depending on both the relative positions and slopes of the two curves, it is possible to determine selectivity, expressed, for example, as the ratio IC_{50} COX-1 : IC_{50} COX-2. The higher the ratio the greater the selectivity for COX-2. However, a high ratio (of say 50 : 1 or even higher) does not guarantee that at clinical dose rates a drug will inhibit COX-2 with no inhibition of COX-1 in vivo.

Based on selectivity, NSAIDs are classified as nonselective, COX-1 selective, or as preferential or selective (denoting increasing degrees of selectivity) for COX-2 (Table 20.13). Validating, confirming, and interpreting the data in Table 20.13 is problematic, as the scientific literature reports widely differing potency ratios (COX-1 :

Table 20.13 COX inhibitor classification[d]

Classification	Example	Comment
Preferential or selective COX-1 inhibitors	Aspirin, ketoprofen (cat)[a], vedaprofen[a], tepoxalin, flunixin (cow)	COX-1 inhibitory potency at least 5-fold greater than COX-2 inhibition
Nonselective COX inhibitors	S-Carprofen(horse)[b], flunixin, ketoprofen(dog, horse)[a], meloxicam[b], phenylbutazone, tolfenamic acid[b], vedaprofen[a]	No significant biological or clinical differences in concentrations producing COX-1 and COX-2 inhibition
Slightly or moderately selective COX-2 inhibitors[c]	S-Carprofen(dog, cat)[b], deracoxib, etodolac etoricoxib, meloxicam[b], tolfenamic acid[b], mavacoxib	COX-2 inhibition potency 5- to 30-fold greater than COX-1 inhibition Some antiinflammatory and analgesic activity may be obtained at concentrations inhibiting COX-2 but not COX-1 At higher concentrations, significant inhibition of COX-1 may occur
Highly or very highly selective COX-2 inhibitors[c]	Cimicoxib, firocoxib, robenacoxib, valdecoxib	More than 50-fold greater potency for COX-2 inhibition Limited inhibition of COX-1 in vivo (normally no GI ulceration or antiplatelet effects) even at maximum therapeutic doses

[a] Differing findings on selectivity from studies in various laboratories. Data in this Table based on whole blood assays.

[b] Species differences in degree of selectivity for COX-2.

[c] Selectivity and specificity depend on position and slope of COX-1 and COX-2 inhibition curves and therefore on the level of inhibition considered e.g., IC_{50}, IC_{80}, IC_{95}, etc.

[d] Ratios of inhibition (COX-1 : COX-2) are markedly affected by experimental conditions, e.g., isolated enzyme versus whole blood assay.

Table 20.14 RS-, S-, and R-carprofen COX-1 : COX-2 IC_{50} and IC_{80} ratios in whole blood assays in four species

Species	Enantiomer	IC_{50} ratio COX-1 : COX-2	IC_{80} ratio COX-1 : COX-2
Human[1]	RS	0.020	0.253
Canine[2]	RS	16.8	101.2
Canine[3]	RS	7	6
Canine[4]	RS	5.4	—
Canine[5]	RS	6.5	—
Canine[6]	S	25.0	—
Canine[6]	R	2.4	—
Equine[6]	S	1.7	—
Equine[6]	R	2.7	—
Feline[5]	RS	5.5	—
Feline[7]	S	25.6	64.9

Data for RS carprofen of dubious value as this is a mixture of two drugs with differing potencies.

[1] Warner et al., 1999.

[2] Streppa et al., 2002.

[3] McCann et al., 2004.

[4] Wilson et al., 2004.

[5] Brideau et al., 2001.

[6] Lees et al., 2004a.

[7] Giraudel et al., 2005a.

Table 20.15 IC_{50} values and IC_{50} COX-1 : COX-2 ratios for S- and R-carprofen enantiomers in the dog and horse (whole blood assays). Source: Data from Lees et al. (2000, 2004a).

Enantiomer	COX-1 IC_{50} (μM)	COX-2 IC_{50} (μM)	COX-1:COX-2 Ratio
Dog			
S(+) carprofen	176	7	25
R(−) carprofen	380	161	2.4
Horse			
S(+) carprofen	25	14	1.7
R(−) carprofen	373	137	2.7

COX-2) for individual drugs, even in a single species. The in vitro experimental conditions impact markedly on the COX-1 : COX-2 inhibition ratios, with higher values (i.e., higher selectivity for COX-2) commonly obtained when isolated enzyme, broken cell, or intact cell determinations in buffer are used in comparison with whole blood assays. The latter are accepted as the "gold standard" as they approximate most closely to conditions in the whole animal. Thus, Gierse et al. (2002) using isolated enzyme assays, reported for deracoxib an IC_{50} COX-1 : COX-2 ratio of 380 : 1 in humans and 1295 : 1 in the dog, whereas McCann et al. (2004) for the same drug, in a canine whole blood assay, obtained a ratio of 12 : 1. Likewise, Ricketts et al. (1998) reported a higher COX-1 : COX-2 ratio for rac-carprofen in isolated cell assays than those obtained in whole blood assays (Table 20.14).

NSAIDs with a chiral center have differing potencies and potency ratios for each enantiomer for COX-1 and COX-2 inhibition. Data for carprofen enantiomers are presented in Table 20.15. Studies using the racemate must be interpreted with great caution.

An additional consideration is the possibility (indeed likelihood) of species differences in NSAID potency for COX-1 and COX-2 inhibition and therefore differences also in COX-1 : COX-2 inhibition ratios. For example, on the basis of whole blood assays, carprofen would be classified as COX-1 selective or preferential in humans, nonselective in the horse, and COX-2 preferential or selective in the dog and cat (Table 20.14). Additionally, there are differences in both potency and potency ratios for the two enantiomers, with S-carprofen being significantly more potent than R-carprofen in the horse and dog. Moreover, in vitro assays indicate the likelihood for carprofen of differing ratios for IC_{50} and IC_{80} inhibition in each of the species cat, dog, and human, because of lack of parallelism between the COX-1 and COX-2 curves. Likewise, for meloxicam in the cat, COX-1 : COX-2 inhibition ratios in a whole blood assay were 3.05 : 1 (50%), 21.4 : 1 (80%), and 192 : 1 (95%) (Giraudel et al., 2005a).

In whole blood canine assays, firocoxib and robenacoxib had IC_{50} COX-1 : IC_{50} COX-2 ratios of 384 : 1 and 140 : 1, respectively (King et al., 2010a) whilst the ratios in the cat were 171 : 1 to 502 : 1 for robenacoxib and 88 : 1 for valdecoxib (Pelligand et al., 2014; Kim et al., 2014). Based on IC_{20}COX-1 : IC_{80}COX-2 ratios, the selectivity of mavacoxib (1.92 : 1) and carprofen (1.95 : 1) in whole blood canine assays was virtually identical (Lees et al., 2015). King et al. (2010a) reported IC_{20}COX-1 : IC_{80}COX-2 ratios in the dog of 0.21 : 1, 0.46 : 1, 2.45 : 1, 5.28 : 1, and 19.8 : 1, respectively, for ketoprofen, meloxicam, S-carprofen, deracoxib, and robenacoxib. Corresponding ratios in the cat were 0.0052 : 1 (ketoprofen), 0.25 : 1 (meloxicam), and 4.23 : 1 (robenacoxib) (Schmid et al., 2010b).

A high level of COX-2 inhibition (80% of I_{max}) may be necessary to obtain clinically desirable levels of antiinflammatory and analgesic activity for most drugs. Integration of in vivo plasma concentration–time data with IC_{80} data for COX-2 generated in whole blood assays in vitro was reported by Giraudel et al. (2005a). They calculated that the recommended dose of carprofen in the cat should produce 50, 80, and 95% inhibition of COX-2 for periods of 72, 57, and 42 hours, respectively, after a single intravenous dose. Corresponding values for the clinically recommended dose of meloxicam in the cat were 23, 9, and 0 hours, respectively. On the basis of COX-1 : COX-2 inhibition ratios and plasma drug concentrations obtained with clinical dose rates, it is likely that (in contrast to the cat) carprofen inhibition of COX-2 is at most moderate in the dog and horse when administered at recommended dose rates, 4.0 and 0.7 mg/kg, respectively. This led to an early suggestion that carprofen may act, in part, by one or more non-COX mechanisms. On the other hand, COX-2 inhibition data suggest that optimal efficacy might be achieved with a lower dose than that recommended in the cat (Giraudel et al., 2005a).

In Vivo and Ex Vivo Determination of COX Inhibition and Correlation with Clinical End-points

Determination of pharmacodynamic parameters for NSAIDs in vivo or ex vivo has the advantage over in vitro methods in that conditions are more directly relevant to physiological/clinical conditions. Using a tissue cage model of inflammation (Higgins et al., 1984) it was suggested that tolfenamic acid, S-ketoprofen, and flunixin are nonselective inhibitors of COX isoforms with only small differences in degree of selectivity between species (Landoni et al., 1995a,b,c; Sidhu et al., 2005, 2006). However, in this model in the cat, ketoprofen was COX-1 selective, while robenacoxib was COX-2 selective; respective IC_{50} COX-1 : IC_{50} COX-2 ratios were 1 : 107 and 67 : 1 (Pelligand et al., 2014). Another example of species differences is provided by phenylbutazone. Lees et al. (2004b) reported that phenylbutazone plasma concentrations 15 times greater in cattle than in the horse produced *less* inhibition of COX-2 in the former species.

Toutain and coworkers (1994) undertook PK-PD modeling of NSAIDs in an equine model of inflammatory joint disease, using clinical indices to predict dosage schedules. Flunixin was more potent (lower IC_{50} values) and efficacious (I_{max}) than phenylbutazone. Concentration–response relationships for stride length for these drugs, and also for meloxicam (Toutain and Cester, 2004) were steep. Simulations predicted, for flunixin for stride length, virtually no response at a dose of 0.5 mg/kg, maximal response persisting up to 10 hours at 1 mg/kg and maximal response up to 16 hours with a dose of 2 mg/kg.

Based on PK-PD modeling and simulations in a model of soft tissue (paw) inflammation in the cat, Giraudel et al. (2005b) calculated dosages of meloxicam required to provide 50, 70, and 90% of maximal responses for a range of indices of pain and inflammation (Table 20.16). In this study, hyperalgesia was not only fully inhibited but was reversed to reveal an hypoalgesic response, in which the threshold for pain sensation exceeded the levels obtained before inflammation induction. Giraudel et al. (2009) reported data in the same feline model for robenacoxib. Average IC_{50} concentrations (ng/ml) corresponding to ED_{50}s were 96 (lameness), 365 (pain), and 371 (antipyresis). These concentrations produced 79–92% COX-2 inhibition and 2.2–6.3% COX-1 inhibition. In a paw inflammation model in the dog, Jeunesse et al. (2011) correlated IC_{50} values for plasma meloxicam concentration with the actions of antipyresis (210 ng/ml) analgesia (390 ng/ml) and vertical force on a force plate (546 ng/ml). These IC_{50} values required 80–90% ex vivo COX-2 inhibition and were associated with levels of COX-1 inhibition of 33–53%. In a urate crystal model of joint disease in the dog, ED_{50} doses of robenacoxib for weight bearing and inhibition of COX-2 were 1.23 and 0.52 mg/kg, respectively (Schmid et al., 2010a).

Table 20.16 Simulated doses of meloxicam required to provide 50, 70, and 90% of maximum responses in the cat in a model of soft tissue inflammation. Source: Data from Giraudel et al., 2005b.

	Meloxicam dose (mg/kg)		
End-point	ED_{50}[a]	ED_{70}[a]	ED_{90}[a]
Skin temperature difference	0.40	0.50	0.67
Pain score	0.30	0.36	0.45
Body temperature	0.24	0.29	0.39
Lameness score	0.26	0.32	0.42
Overall locomotion variable	0.28	0.33	0.43

[a] ED (mg/kg) is the meloxicam dose producing 50, 70, or 90% of the maximum possible average drug response that can be obtained with a single subcutaneous administration of meloxicam. The manufacturer's recommended dose is 0.3 mg/kg.

For 2-arylpropionate NSAIDs, S-enantiomers are generally more potent COX inhibitors than R-enantiomers. However, both enantiomers were claimed by one author to be analgesic (Brune et al., 1992). Moreover, in the pig, mechanical nociceptive testing in a kaolin-induced lameness model indicated a higher potency for R-ketoprofen (IC_{50} = 1.6 μg/ml) than for S-ketoprofen (IC_{50} = 26.7 μg/ml) (Fosse et al., 2011).

Lees et al. (2015) compared in vitro canine IC_{20}COX-1 (2.46 μg/ml) and IC_{80}COX-2 (1.28 μg/ml) for mavacoxib in whole blood assays with maximum and minimum steady state concentrations in typical clinical OA cases of 2.08 and 1.28 μg/ml, respectively, indicating that peak concentration did not exceed IC_{20}COX-1, while trough concentration was equal to IC_{80}COX-2. Similar correlations between mavacoxib IC_{80}COX-2 and clinical end-points were established in canine models of synovitis (Lees et al., 2015).

Central Sites and Mechanisms of Action of NSAIDs

NSAIDs exert central as well as peripheral actions (Dolan and Nolan, 2000). COX-1 and COX-2 are expressed constitutively in dorsal root ganglia, in spinal dorsal and ventral grey matter, and in nonneuronal cells including astrocytes (Svensson and Yaksh, 2002). Central COX-2 up-regulation, in response to inflammation at peripheral sites, leads to release of PGE_2, which lowers spinal depolarization thresholds, thereby increasing action potentials and repetitive spiking. PGE_2-induced neuronal effects involve central sensitization (comparable to that occurring peripherally) or "wind-up" (Malmberg and Yaksh, 1992). Thermal hyperalgesia induced by injection of carrageenan into the paw was suppressed by both intrathecal and systemically administered COX-2 selective drugs and by a COX-1 selective inhibitor administered systemically (Yaksh et al., 2001). Moreover, intraspinal administration of a COX-2 inhibitor decreased central PGE_2 concentrations and suppressed mechanical hyperalgesia (Samad et al., 2001). Thus, the central actions of nonselective and COX-2 selective NSAIDs contribute to their antihyperalgesic actions.

Additional Actions of NSAIDs

As well as inhibition of COX isoforms, NSAIDs possess many other actions at the molecular level (Table 20.17). These include inhibitory effects on signal transduction pathways (Weissmann, 1991). Several NSAIDs, including phenylbutazone and flunixin, inhibit leukocyte migration in in vitro assays (Dawson et al., 1987) but not in vivo. Likewise, actions such as inhibition of NFκB demonstrated in vitro with high concentrations (Bryant et al., 2003) may or may not occur with therapeutic concentrations in vivo.

What is not known is the extent (if any) to which these additional actions contribute to the therapeutic effects of NSAIDs, when administered at clinical dose rates and by recommended routes, as many literature reports describe in vitro studies in which drug concentrations may be classed as heroic. Nevertheless, some in vitro data do correlate with in vivo findings. For example, carprofen has been shown in vitro to stimulate the synthesis and retard the breakdown of cartilage matrix molecules, proteoglycans, by both chondrocytes and cartilage explants in culture (Benton et al., 1997; Armstrong and Lees, 1999b; Frean et al., 1999). This might explain some beneficial effects of carprofen in canine OA (Pelletier et al., 2000). However, the effects of carprofen enantiomers on cartilage proteoglycan synthesis were concentration dependent, higher concentrations inhibiting synthesis. Similarly, Brandt (1991) reported that salicylate inhibited cartilage proteoglycan synthesis in vitro.

Acetaminophen differs from other NSAIDs in acting principally centrally rather than peripherally. However, Aronoff et al. (2005) have demonstrated a peripheral action – on the peroxidase enzyme component of prostaglandin H_2 synthase; the latter comprises peroxidase and COX portions. Other mechanisms of action of acetaminophen have been proposed, including activation of descending inhibitory pain pathways. Acetaminophen is not antiinflammatory (at clinical dose rates) but is analgesic and safe in the dog at a dosage of 15 mg/kg administered every 8 to 12 hours (Papich, 2008).

Toxicity of NSAIDs

General Considerations

The capacity for synthesis of PGs is common to almost all cell types; they are ubiquitous compounds, associated with a range of physiological and pathophysiological functions, inhibition of which is the main basis for

Table 20.17 Possible mechanisms of action of NSAIDs

Inhibition of Cyclooxygenase in the Arachidonic Acid Cascade Leading to Blockade of Synthesis of Proinflammatory Mediators is the Principal Mechanism of Action	
Additional actions of some drugs	**Examples**
Inhibit 5-LO	Licofelone, tepoxalin
Inhibit prostanoid release from cells	2-arylpropionic acids, indomethacin
Inhibit IκB kinases or NFκB to block COX expression	Aspirin, carprofen, flunixin, indomethacin, diarylheterocycles (COXIBs)
Inhibit the actions of eicosanoids on their receptors	Fenamates e.g., tolfenamic acid
Inhibit the actions of bradykinin	Flunixin, ketoprofen, tolfenamic acid
Modulate the release of proinflammatory cytokines, e.g., IL-1, IL-6, TNF-α	Carprofen (IL-6), tepoxalin (IL-1, IL-6, TNF-α)
Stimulate nuclear receptors, e.g., PPAR-γ	Salicylates, 2-arylpropionic acids, arthranilic acids, indene acetic acids
Increase intracellular breakdown of ATP to adenosine	Salicylates
Modulate the synthesis of nitric oxide	Several drugs
Inhibit neutrophil chemotaxis and/or chemokinesis[a]	Vedaprofen, salicylates
Inhibit neutrophil activation, thereby preventing (a) Release of oxygen radicals e.g., superoxide (b) Release of both lysosomal and nonlysosomal enzymes	Flunixin, ketoprofen, piroxicam, tolfenamic acid
Increased synthesis and reduced breakdown of cartilage matrix (proteoglycans)	Carprofen

[a] Most NSAIDs inhibit leukocyte movement in a concentration-dependent manner in *in vitro* assays (Dawson et al., 1987) but clinically recommended doses *in vivo* usually do not inhibit leukocyte chemotaxis or chemokinesis.

NSAID toxicity as well as therapeutic effects. The most frequent and clinically significant side effects are the effects on the GI. The principal target organs and tissues for *potential* toxicity are:

- gastrointestinal irritation associated with vomiting (possibly blood stained) in monogastric species, ulceration and erosions, leading to plasma protein losing enteropathy and melena;
- renotoxicity including occasional acute renal failure;
- hepatotoxicity (cholestastic or parenchymal);
- inhibition of hemostatic mechanisms leading to hemorrhage;
- blood dyscrasias;
- delayed parturition;
- delayed soft tissue healing;
- delayed fracture healing.

It must be emphasized that clinical manifestations of toxicity do not occur in the great majority of animals receiving recommended dose rates of NSAIDs. It is in the nature of pharmacovigilance data that a proportion of adverse reaction reports will be incidental and not drug related, whilst some are due to overdosing or reflect drug interactions. On the other hand, a proportion of reactions to NSAIDs will not be reported. Therefore, it is important to: (i) consider the incidence (low) and severity (usually mild and/or transient) of side effects; and (ii) distinguish between side effects actually reported in clinical use and those postulated on the basis of the molecular actions of NSAIDs or predicted from experimental animal studies.

An important consideration for animals is the ready (over-the-counter) availability of certain NSAIDs to animal owners through pharmacies and other retail outlets. A possible consequence is severe toxicity, when poorly informed owners have limited knowledge of NSAID pharmacological and toxicity profiles. For example, in the cat the metabolite of aspirin (salicylate) is cleared slowly and has a long elimination half-life (Table 20.6), so that approximately *one-tenth* of a 300-mg tablet represents a *maximum* dose, which should not be administered more frequently than 48-hour intervals. Likewise, the cat converts acetaminophen (paracetamol) to toxic metabolites and should never be given to this species (Hjelle and Grauer, 1986). However, Papich (2008) has suggested that acetaminophen might be reevaluated for use in the dog. This would seem to be appropriate in that peripheral tissues such gastric and duodenal mucosae and the kidney are spared its toxicity. There was no apparent toxicity in the dog at single dosages of less than 100 mg/kg (Savides et al., 1984).

In light of the potential toxicity of NSAIDs, avoidance of use is recommended in animals which are: dehydrated/ hypovolaemic/ hypotensive; avidly retaining sodium (congestive heart failure, hepatic cirrhosis); have existing renal, hepatic, or cardiovascular conditions; have gastric ulcers, reduced gastric blood flow; and animals receiving some other drug classes, including corticosteroids (delay wound healing) and aminoglycosides and polymixins (potential for nephrotoxicity). Drug interactions have been reported in humans or in experimental animal studies: increased risk of convulsions with fluoroquinolones; inhibition of the hypotensive actions of beta-blockers; inhibition of the actions of furosemide and angiotensin converting enzyme inhibitors. Delayed clearance, longer half-life, and increased plasma concentrations in neonates are possible and this in turn might lead to toxicity unless dosage regimens are reduced, though data in this field are limited.

There has been much discussion on the length of a wash-out period when one NSAID is substituted for another (Papich, 2008). Providing the switch is being made for nontoxicological reasons (this would usually be poor clinical response), it is probably in general acceptable to commence dosing with the new drug after 24–48 hours, since for most drugs a once-daily dosing schedule is required to maintain therapeutic responses. For mavacoxib at least a 28 day wash-out interval is recommended, because of the long elimination half-life. With this exception, the available data do not support withholding the replacement drug for up to 7 days for animals in pain on ethical grounds (Papich, 2008). However, if the substitution is made because of actual or suspected toxicity of the first drug, an interval of five to seven half-lives might be appropriate to allow for elimination of some 97% of the drug and healing of damaged tissue.

Extensive preclinical safety studies in healthy animals and tolerability in clinical trials have established generally good profiles for COXIBs, although the data do not always indicate superiority to the classical nonselective COX inhibitors. In preclinical placebo-controlled studies, the administration of doses of mavacoxib and robenacoxib several-fold greater than clinical doses for several weeks or months have indicated low toxicity (King et al., 2010b, 2011; Lees at al. 2015). Whilst the ideal would be to conduct placebo-controlled clinical trials to establish tolerability, these are rarely conducted for good ethical reasons.

Gastrointestinal Toxicity

Reports of serious GI-related side effects of NSAIDs in clinical use are rare, but mortality can occur. This normally involves marked GI ulceration and erosion and loss of blood or plasma into the peritoneal cavity, with sufficient fluid loss to cause hypovolemic shock. Moreover, through disruption of the mucosal GI barrier, the GI microflora may gain access to the circulation, leading to endotoxic shock. NSAID-induced irritation in the stomach can lead to persistent emesis, with associated fluid losses, requiring therapy with proton pump inhibitors or selective H_2-receptor antagonists. Alternatively, misoprostol, a stable derivative of PGE_1, has been used in combination with NSAIDs.

The GI toxicity of NSAIDs is due, in part, to "local actions" on the stomach. For aspirin, this can arise from its low water solubility and the creation of a local hypertonic solution bathing the stomach mucosa. However, the irritant actions of NSAIDs can occur throughout the tract and after parenteral as well as oral administration. Ulcerogenic doses of NSAIDs reduce PG concentrations in the gastric mucosa leading to reduced production of bicarbonate-rich mucus secretion, which provides a protective, viscous, alkaline lining fluid. These effects of NSAIDs have been linked to inhibition of the cytoprotective effects of COX-1. COX-1/PG gastroprotection also involves decreased volume and acidity of gastric secretion and local vasodilation. However, knock-out mice in which the COX-1 gene has been deleted fail to develop mucosal ulcers. Moreover, in the rat, neither SC-540 (a selective COX-1 inhibitor) nor celecoxib (selective for COX-2) alone caused ulcers, but erosions were induced when they were given in combination (Wallace et al., 2000).

COX-2 expression is increased on the edges of gastric ulcers; leading to the synthesis compounds, which accelerate ulcer healing, possibly by increasing angiogenesis via inhibition of cellular kinase activity and upregulation of vascular endothelial growth factor in the gastric mucosa. There may be roles for both PGE_2 and $15dPGJ_2$ in ulcer healing. COX-2 inhibitors have been shown experimentally to delay ulcer healing (Bataar et al., 2002) but this seems not to be a significant problem clinically.

A role for neutrophils in NSAID-induced GI toxicity has been proposed; NSAIDs increase neutrophil adherence to the mucosal vascular endothelium. In addition, monoclonal antibodies, which block leukocyte adhesion, reduce the severity of NSAID-induced gastropathic effects. The gastropathy is also reduced in animals that are neutropenic. Neutrophil adherence to endothelium may contribute to mucosal injury by releasing oxygen-derived free radicals, proteases, and other compounds. Moreover, neutrophil adherence might cause capillary obstruction, resulting in reduced gastric mucosal blood flow and hypoxia. However, not all data support a role for neutrophils in NSAID-induced GI ulceration.

Another theory of NSAID-induced gastropathy implicates reduced formation of the free radical gas NO. There is reduction or abolition of gastric damage by NSAIDs when they are used as nitrosoesters (CINODs). The nitroso group acts as a NO donor in vivo. The increased leukocyte adherence to the mucosal vascular endothelium caused by NSAIDs does not occur with the nitroso derivatives. Additionally, released NO may, through its vasodilator action, increase gastric and intestinal mucosal blood flows directly, thereby inhibiting NSAID-induced ischemia and hypoxia. Another proposed basis for NSAID-induced gastrotoxicity is diversion of substrate (AA) towards LO enzymes and the formation of products adversely affecting gastric mucosal integrity. Clearly, many factors may be involved.

An approach commonly employed to minimize GI irritation is to administer NSAIDs in feed or with water, to reduce drug concentration in contact with the GI mucosa. In addition, the site of NSAID-induced GI ulceration in the horse involves a specific interaction with feed. When administered with hay or horse nuts, phenylbutazone and flunixin bind to feed (Lees et al., 1988b). This limits absorption from the upper GI, as binding to feed is of the order of 98 and 70%, respectively. When the fibrous component of feed is partially digested in the large intestine, bound drug is released and becomes available not only for delayed absorption but also to exert a local irritant action. This probably explains the high incidence of lesions in the distal parts of the GI when high doses of phenylbutazone are administered to horses (Snow et al., 1979; Lees et al., 1988b).

Early reports described extensive erosions throughout the GI associated with a protein losing enteropathy and high mortality in ponies receiving oral phenylbutazone and ketoprofen doses only moderately in excess of those then recommended for clinical use (Snow et al., 1979; Lees et al., 1983; Taylor et al., 1983; MacAllister et al., 1993). There is a steep dose–effect relationship for the toxicity of phenylbutazone in the horse. Reports that ponies are more susceptible to the toxic effects of phenylbutazone than larger equine breeds have not been confirmed. Other manifestations of phenylbutazone toxicity in the horse include neutropenia, bone marrow suppression, parenchymal hepatotoxicity, and necrotizing phlebitis of the portal vein (Lees et al., 2003; Murray, 1985). Oral dosing of flunixin for 5 successive days at the recommended dose rate also reduced plasma total protein and albumin significantly in the horse (Lees and Higgins, 1985). Carprofen, on the other hand, has a wider safety margin in this species.

Using clinically recommended dose rates in the dog, the greatest incidence and severity of GI-related side effects were reported for flunixin and ketoprofen, while carprofen and firocoxib were better tolerated (Luna et al., 2007; Steagall et al., 2007). Carprofen is less likely than many other available agents to cause GI irritation in the horse and dog (Lees et al., 2004a), possibly arising from its selectivity for COX-2. It is likely that improved GI tolerance is also a property of COXIBs (cimicoxib, deracoxib, firocoxib, mavacoxib, parecoxib, and robenacoxib). Possible additional factors contributing to good GI tolerance of COXIBS are very short half-life plus rapid clearance from the central compartment (robenacoxib), once-monthly dosing, plus limited daily exposure from biliary secretion (mavacoxib) and inactivity of the parent drug (parecoxib converted to valdecoxib).

Overall, the human literature indicates a better GI safety profile for selective COX-2 inhibitors in comparison with nonselective inhibitors (Lanza et al., 1999; Hawkey et al., 2000) and valdecoxib and lumiracoxib provided similar levels of gastroduodenal tolerability to placebo treatments (Goldstein et al., 2003; Rordorf et al., 2003). However, in both humans and animals, COX-2 selective drugs are not free of GI-related side effects at high dose rates. For example, for firocoxib in healthy dogs at five times the recommended daily dose for 3 months, toxic signs included inappetence, emesis, duodenal ulcers, lipid accumulation in the liver, and vacuolization in the brain. However, emesis and duodenal ulcers did not occur with three times the recommended dose, administered daily for 6 months. Puppies aged 10–13 weeks were more susceptible than dogs aged 6–12 months (Steagall et al., 2007). Field trials in dogs reported a GI safety profile for mavacoxib equal to or possibly better than carprofen (Payne-Johnson et al., 2015).

Despite their potential for GI toxicity, NSAIDs as a group have generally good safety profiles, when administered at recommended dose rates in clinical subjects (KuKanich et al., 2012). Gastric adaptation in dogs may account at least partially for this (Papich, 2008). Components of this adaptation include increased gastric blood flow, reduced inflammatory cell infiltrate, and increased mucosal cell regeneration. When GI ulceration has occurred from any cause, COX-2 is rapidly induced; the enzyme generates cytoprotective prostaglandins. As predictable, COX-2 inhibitors inhibit the healing of preexisting ulcers in experimental rodent and canine studies. Selective COX-2 inhibitors are therefore unlikely to cause ulcers at recommended dose rates but they might delay the healing of preexisting ulcers (Wooten et al., 2008, 2010). Further studies are required.

Local Irritancy

Some NSAIDs may cause tissue necrosis after injection by nonvascular routes, for example phenylbutazone is formulated as a high-strength solution, whereas other NSAIDs such as carprofen and meloxicam have been specifically formulated for intramuscular administration.

Renal toxicity

Nephrotoxic effects of NSAIDs at recommended dose rates do not generally occur in healthy normotensive animals with free access to drinking water. Thus, pharmacovigilance studies indicated a very low incidence of nephrotoxicity in the dog for carprofen and mavacoxib; more than 800 dogs for the latter drug with treatment for 6.5 months (Payne-Johnson et al., 2015). In healthy cats, neither tolfenamic acid nor vedaprofen, administered once daily for 14 days, produced any demonstrable renotoxicity. However, PGs play subtle (but key) roles in renal physiology, regulating glomerular filtration, renin release, and tubular sodium reabsorption. Both isoforms of COX are present at several intrarenal sites with some differences between species, the functional significance of which is largely unknown. In the dog, COX-2 is expressed constitutively in the macula densa, thick ascending limb of the loop of Henle and interstitial cells, and also in humans in glomerular podocytes and the afferent arteriole. Chapter 24 should be consulted for details about renal pharmacology. COX products regulate renal homeostatic mechanisms in three main ways: glomerulotubular feedback to stabilize glomerular filtration rate (GFR); response to reduced water intake or increased water loss; and response to increased sodium intake. Potential side effects of NSAIDs include sodium retention (with accompanying edema), reduced GFR, and systemic hypertension.

Three aspects of the role of COX in the kidney, in relation to the use of NSAIDs, deserve particular consideration. First, COX products (possibly both COX-1 and COX-2 derived) are involved in maintaining renal blood flow and GFR in the face of reduced arterial pressure and/or fluid and sodium depletion, a phenomenon termed autoregulation. The macula densa (distal tubule cells which abut the afferent arteriole) senses high concentrations of chloride and secretes a signal (probably adenosine) to constrict the afferent arteriole and reduce GFR. When the macula densa senses low chloride concentrations, it secretes PGE_2, PGI_2, and nitric oxide, and these signal to modified afferent arteriole cells (juxtaglomerlular apparatus) to secrete renin, which leads to angiotensin I then angiotensin II synthesis, which in turn constricts the efferent arteriole, increasing GFR. Angiotensin II leads further to aldosterone secretion from the adrenal cortex and this hormone promotes sodium retention and potassium secretion. General anesthesia is a particular circumstance in which hypotension may arise from the cardiovascular actions of premedicant, induction, and maintenance agents, and this may be compounded by losses of blood and insensible water losses during surgery.

Second, under conditions of water deprivation, COX-2 in the renal medulla is up-regulated. If, under water deprivation conditions, NSAIDs are administered, interstitial cells undergo apoptosis, potentially leading to renal papillary necrosis. Third, the thick ascending limb of the loop of Henle transports some 25% of filtered sodium. These cells express COX-2 and release PGE_2. Feeding high concentrations of sodium up-regulates COX-2 in the medullary portion of the thick ascending limb. Administration of NSAIDs blunts the normal natriuretic response, with increased tendency to edema formation. However, the clinical relevance of this mechanism in veterinary medicine is unknown.

Blockade of the renoprotective actions of intrarenally released PGs explains the nephrotoxic effects of NSAIDs, and there were several early reports of acute renal failure with recommended doses of drugs such as flunixin, following recovery from anesthesia in the dog. Some authorities recommend restriction for some NSAIDs to postoperative administration, when blood pressure has been restored to normal and fluid losses have been replaced. Despite these considerations, an experimental study in healthy beagle dogs revealed no adverse effects on renal function of oral meloxicam, when the animals had been rendered hypotensive during anesthesia (Bostrom et al., 2006). Likewise, carprofen did not alter renal function in healthy dogs anesthetized with propofol and isoflurane (Ko et al., 2000). However, Forsyth et al. (2000) reported 26–34% reduction in GFR after surgery (routine castration) in dogs premedicated with carprofen or ketoprofen and then anesthetized with isoflurane. Oral daily administration of vedaprofen and tolfenamic acid in healthy cats produced no demonstrable renotoxicity (Khwanjai et al., 2012). Reports of renal papillary necrosis in horses receiving clinical dose rates of phenylbutazone or flunixin appeared in the 1980s, when horses on NSAID therapy also had restricted access to water.

Murine knock-out studies suggested that COX-2 is crucially involved in kidney maturation (Dinchuk et al., 1995). There is therefore potential concern on the use of NSAIDs in pregnancy as well as in neonates. In addition, very young animals are likely to have immature mechanisms of elimination (both renal and hepatic) of drugs, including NSAIDs. Therefore, in physiological or pathological conditions of hyponatremia, fluid depletion, and hypotension, and in disease states such as diabetes mellitus and diminished adrenocortical function, COX isoforms may maintain renal perfusion, GFR, and tubular function. All NSAIDs have the potential, in these conditions, to cause edema, reduced GFR, hyperkalemia, and hypertension. Moreover, in man, rofecoxib interfered with antihypertensive drugs, such as ACE inhibitors, angiotensin receptor blockers, and β-adrenoceptor blockers, which exert their actions, in part, through the renal vasodilator actions of PGs. Similarly, NSAIDs can interfere with the actions of loop diuretics, such as furosemide ; chronic treatment of dogs with furosemide plus carprofen or etodolac produced a reversible reduction in GFR (Surdyk et al., 2012). Potential toxicity from the combined use of NSAIDs with known nephrotoxic drugs, such as aminoglycoside and polymixin antimicrobial drugs, should be borne in mind. Moreover, dogs and cats developing chronic kidney disease are commonly elderly with multiple problems, including OA, requiring NSAIDs for pain relief. Particular care may be required in such patients, although definitive epidemiological data are lacking. Up-regulation of COX-enzymes in cats with chronic kidney disease in all areas of the kidney examined has been demonstrated (Suemanothan et al., 2009) but there was no association between proteinuria or progressive kidney disease and the magnitude of COX enzyme expression, suggesting that COX enzyme up-regulation may not be linked to progressive kidney disease in the cat.

Hepatotoxicity

Liver toxicity is rare in animals treated with clinical dose rates of NSAIDs. However, parenchymal hepatotoxicity has been described for phenylbutazone in the aged horse (Lees et al., 1983), possibly as a consequence of increased blood levels in older subjects (Lees et al., 1985). Cholestatic toxicity has also been described in the horse treated with phenylbutazone. Acute hepatic necrosis is a rare complication of carprofen in the dog, with a likely predisposition of the Labrador and related breeds (MacPhail et al., 1998). When therapy was discontinued in the dog, resolution of biochemical-associated liver changes and clinical signs occurred. An immune-based mechanism has been postulated. Hepatocellular damage in dogs has been reported with acetaminophen overdose.

Cardiovascular System and Blood Cells

The principal eicosanoids with local hormonal roles on blood vessels and blood cells are TXA_2 (a vasoconstrictor, proaggregatory agent, which is released from platelets and involved in blood clotting) and PGI_2 (a vasodilator, antiaggregatory, antiplatelet adhesive agent, released from endothelial cells, which prevents clotting in normal circulatory function). Platelet COX-1 is constitutive and endothelial cell COX comprises both isoforms. The TXA_2/PGI_2 system maintains a homeostatic balance, which can be disrupted by selective COX-2 inhibitors, potentially causing increases in blood pressure (through removal of the vasodilator influence) and a tendency toward clotting (through loss of the antiaggregatory effect). The potential for COX-2 inhibition to cause “cardiovascular events,” including thrombus formation and myocardial infarction, which may be life threatening, has been the subject of considerable debate in human medicine. Even in man, however, for whom very large-scale clinical trials have been undertaken, the situation is unclear. While several studies indicate an increase in relative cardiovascular risk in patients receiving selective COX-2 inhibitors, adverse cardiovascular effects are generally not apparent until continuous treatment has been given for periods of 18 months or longer. Furthermore, in one study, the greater relative risk with rofecoxib compared to naproxen was ascribed to a possible protective action of naproxen (which may be a preferential COX-1 inhibitor) rather than deleterious effects of the COX-2 inhibitor.

The adverse cardiovascular effects of selective COX-2 inhibitors in humans are dose related and there may be no significant increase in relative risk at low dose rates (Ray et al., 2002). Moreover, some studies have shown an increase in relative risk for cardiovascular effects for nonselective NSAIDs. Others have failed to demonstrate any increase in risk; a metaanalysis of eight OA trials indicated similar rates of thrombotic cardiovascular events with placebo, nabumetone, rofecoxib, and comparator nonselective NSAIDs, which included ibuprofen and diclofenac (Reicin et al., 2002).

The relevance of these human studies for veterinary species is unclear. The dog (but not the cat) is relatively resistant to thromboembolic diseases. Therefore, while the potential for adverse cardiovascular effects in veterinary patients must be borne in mind, veterinary experiences indicate a risk–benefit relationship in favor of benefits.

Among NSAIDs, aspirin is a special case, because of its irreversible action and the inability of platelets, being anuclear, to synthesize new COX-1. The potential for inhibition of hemostasis and prolonged bleeding time must therefore be borne in mind when aspirin is used therapeutically, even at low dose rates, and when other NSAIDs are used at high dose rates. In the horse, aspirin's antiplatelet action is manifested by virtually complete inhibition of TXA_2 synthesis for up to 1 week after a single dose, yet the terminal half-life after intravenous dosing is only 9 minutes (Lees et al., 1987a). This prolonged action might be due to inhibition of COX-1 in bone marrow megakaryocytes as well as circulating platelets. In humans, there is concern that concomitant administration of aspirin (for its antithrombotic effects) and other NSAIDs (for their analgesic, antiinflammatory effects) may result in interference with aspirin's antiplatelet actions (MacDonald and Wei, 2003). This might occur as a consequence of the very short half-life of aspirin, combined with NSAID prevention of aspirin entry to the COX-1 active site. In the dog, phenylbutazone was linked in early studies to blood dyscrasias (Tandy and Thorpe, 1967; Watson et al., 1980). It is not known whether these were dose-related or idiosyncratic, but blood dyscrasias are also associated with high doses of phenylbutazone in the horse. Early studies indicated marked depression of the bone marrow in cats receiving aspirin or phenylbutazone; in retrospect, it seems likely that the doses used were much greater than those required for clinical efficacy and may represent the effects of gross overdosage (Larson, 1963; Carlisle et al., 1968).

Acetaminophen has good GI tolerance but is toxic to the cat, in which species it is metabolized to oxidative compounds with cytotoxic properties. The metabolites overwhelm the glutathione scavenging system, resulting in methemoglobinemia and, less commonly, centrolobular hepatic necrosis. Recommended antidotes are antioxidants, such as vitamin C or *N*-acetylcysteine (St Omer and McKnight, 1980). In addition, the H_2-receptor antagonist, cimetidine, a hepatic microsomal enzyme inhibitor, has been recommended to inhibit formation of the toxic metabolites (Jackson, 1982). Similar signs of toxicity may occur with high dose rates of acetaminophen in the dog (Hjelle and Grasser, 1986).

Bone, Tendon, and Ligament Healing

In COX-2 null mice fracture healing is impaired (Zhang et al., 2002). However, the roles of PGs in bone metabolism are complex; PGE_2, PGI_2, and TxA_2 first stimulate new bone formation by osteoblasts and resorption by osteoclasts through EP_2 and EP_4 receptors (Kawaguchi et al., 1995). Moreover, in response to particulate wear debris, COX-2 increased the concentration of several proinflammatory cytokines (TNF-α, IL-lβ, IL-6) and other osteotrophic factors. PGs are thus involved in inflammatory bone conditions, with release by osteoblasts following injury (Gajraj, 2003; Gerstenfeld et al., 2003).

COX-1 is present constitutively in osteocytes, osteoblasts, and osteoclasts, but it is COX-2 mRNA levels that are raised and PGE_2 synthesis increased in the early stages of bone healing. Rodent studies have demonstrated reduced fracture healing in response to nonselective and COX-2 selective COX inhibitors (Simon et al., 2002; Goodman et al., 2003). These effects are reversible if dosing is not prolonged.

COX-2 is up-regulated only during the initial stages of bone healing and various orthopedic inflammatory and neoplastic conditions, such as canine and human osteosarcoma and canine pulmonary osteosarcoma (Radi and Khan, 2005). Reports of impairment of bone, tendon, and ligament healing by NSAIDs are conflicting, with some studies failing to show any deleterious effects. Radi and Khan (2005) concluded that, in animal models, NSAIDs retarded bone, ligament, and tendon healing, but the effects were confined to the early stages of healing, with no significant impact on long-term outcome. Indeed in one study, both COX-2 selective and nonselective inhibitors improved mechanical strength in the later phase of healing (Riley et al., 2001). There is a dearth of published clinical data in veterinary medicine in this field.

Soft Tissue Healing and Repair

There is potential for NSAIDs to affect the healing of soft tissues, given the postulated role of COX-2 products in the resolution phase of acute inflammation. Experimental data in mice both for (Laudederkind et al., 2002) and against (Blomme et al., 2003) a role for COX-2 in dermal wound healing have been presented. Data on wound healing relevant to species of veterinary interest are lacking.

Respiration

A subset of human asthmatic patients display signs of respiratory distress when administered aspirin (and in some subjects other NSAIDs also). This may be due to diversion of the substrate, AA, towards 5-LO and the increased synthesis of bronchoconstrictor LTs.

Skin

Occasional skin reactions to NSAIDs, including urticaria, rashes, and angioedema, have been reported. The mechanisms are unclear but, in some instances, a hypersensitivity reaction may be involved and possibly related to the high degree of binding to plasma proteins.

Reproductive System

Prostanoids are involved in ovulation, fertilization, and blastocyte implantation (Kniss, 1999). This is fully covered in Chapter 27 of this text. COX-2 null mice do not ovulate, and fertilization, implantation, and decidualization do not proceed normally. In early pregnancy, COX-2 is present in the uterine epithelium and is likely to be involved in ova implantation, angiogenesis, and labor. It has been suggested that COX-1 mediates the early stages of labor onset, involving uterine contractions and early stages of cervix dilation, whereas COX-2, induced by cytokines from the decidua, trophoblast, or fetal membranes, generates prostanoids to sustain myometrial contractions and cervical ripening leading to fetal expulsion. Experimental studies have indicated a role for PGE_2 in maintaining in utero the patency of the ductus arteriosus, and treatment with COXIBs postpartum leads to premature closure. Based on these roles of COX, various effects might be predicted from dosing with NSAIDs. In the testes, COX-2 is the main isoform and PGs formed include $PGF_{2\alpha}$, PGE_2, and PGD_2. The effects of NSAIDs on testicular function have not been extensively studied.

Nervous System

COX-2 is present constitutively, particularly in neonates, in neuron cell bodies and dendrites at several brain sites. Centrally, PGs are involved in body temperature regulation, hyperalgesia, and neuron development. PGs are not neurotransmitters but they modulate nerve transmission. In view of these roles of COX in CNS functions, it is perhaps surprising that so few significant central side effects are associated with NSAID use.

Therapeutic Uses of NSAIDs

Over the last two decades, the number of NSAIDs approved by regulatory bodies has increased significantly. This has been matched by a considerable increase in the extent of usage, driven by recognition of the wide range of circumstances in which the control of inflammation and pain is desirable for medical, welfare, and economic reasons. Products are available in both oral (tablets or liquids) and parenteral formulations. For some drugs, treatment may be continued for many months. Almost invariably, the dose interval is 24 hours. The main exception is mavacoxib, which is administered orally with an interval of 14 days between first and second doses and at 28 day intervals up to 6 months thereafter. This dosage schedule should improve dosing compliance by owners. Possible consequences of daily peaks and troughs of concentration (most NSAIDs) compared to sustained COX-2 inhibition throughout the interdose interval (mavacoxib) have been discussed (Lees et al., 2015).

Acute Pain and Inflammation

In animals, the presence of pain and its intensity can be difficult to recognize and quantify. Nevertheless, it is now acknowledged that animals “feel” pain (physiological) and we must assume that they also “suffer” pain (psychological) as do humans. Therefore, both subjective (e.g., semiquantitative scoring systems and visual analogue scales) and more objective (e.g., force plate) indices of pain/lameness have been used (Flecknell and Waterman-Pearson, 2000; Holton et al., 1998; Lipscomb et al., 2002). There is now widespread use of NSAIDs in all major veterinary species: (i) perioperatively, (ii) to treat acute trauma (e.g., road accident cases and equine/canine sports injuries), (iii) for a range of acute and chronic musculoskeletal conditions, and (iv) to treat the severe pain associated with colic in the horse (Balmer et al., 1998; Lascelles et al., 1995, 1998; Welsh et al., 1997; Robertson, 2005). In the latter condition, NSAIDs can mask the signs of colic, so that diagnosis of the underlying cause should be made prior to dosing. NSAIDs are used extensively to control acute pain associated with surgical and medical conditions (Table 20.18).

For the control of postoperative pain, NSAIDs may be as effective as opioids (Lascelles et al., 2007). However, NSAIDs are generally longer acting, do not possess the central nervous depressant/dependence side effects of opioids, and are subject to fewer legal restrictions. They are, therefore, commonly used as alternatives to opioids

Table 20.18 Conditions that may cause severe pain for which NSAID usage should be considered

Surgical conditions	Medical conditions
Orthopedic surgery	Bone tumors
Aural surgery	Disc lesions
Oral surgery	Otitis externa
Anal surgery	Various ophthalmic conditions
Upper abdominal surgery	Pancreatitis
Onychectomy	Acute moist dermatitis
	Equine colic

or to extend the duration of analgesia initiated by opioids. Preemptive analgesia with both drug classes (often in combination in multimodal drug approaches) has been widely advocated (Welsh et al., 1997). It involves administration pre- or early intraoperatively, both to enhance surgical conditions and also to control pain postoperatively (Lascelles et al.,1998). NSAIDs used preemptively include carprofen, meloxicam, tolfenamic acid, and the COXIBs. The preemptive concept derived from experimental studies in rodents but its benefit for NSAIDs in veterinary patients is less clear, although it is well supported by one study (Lascelles et al.,1998). The use of NSAIDs perioperatively can have the additional advantage of reducing edematous swelling and thus preventing wound breakdown. However, antiedematous doses, may be higher than those required for analgesia.

There is concern that the administration of NSAIDs before recovery from anesthesia might be associated with low incidence but life-threatening acute renal failure (see Section Renal toxicity). Of the agents in veterinary use, carprofen, meloxicam, and the COXIBs may be the agents of choice for pre- and intraoperative use. In clinical trials in the dog at recommended dose rates, analgesia with firocoxib was equal to or greater than that of carprofen or etodolac. For the control of pain and inflammation associated with both soft tissue and orthopedic surgery, robenacoxib was noninferior to meloxicam and had good tolerability in the dog (Gruet et al., 2011, 2013). For the control of postoperative pain in the cat, robenacoxib was superior to placebo (King et al., 2012) and superior to meloxicam for mainly soft tissue surgery (Kamata et al., 2012). For the treatment of pain and inflammation associated with musculoskeletal disorders in cats, robenacoxib was either noninferior or superior to ketoprofen, depending on the end-point monitored and both drugs displayed good tolerability (Giraudel et al., 2010; Sano et al., 2012).

Chronic Pain

NSAIDs are used to treat chronic pain, associated, for example, with the arthritides and cancer. In the horse and dog, OA is the most common indication for NSAIDs, to control short term flare-ups or for continuous management (Sanderson et al., 2009; Innes et al., 2010). Potential benefits are reduced pain, improved mobility and joint stability, reduction in muscle atrophy, and possibly reduced rate of disease progression. Many mediators are involved in joint conditions, leading to inflammation and increased angiogenesis of the synovial lining. The inflammation is particularly acute in canine rheumatoid arthritis compared to OA, and in OA acute inflammation is a more prominent component in the dog than in the horse. Some authors therefore prefer the term degenerative joint disease (DJD) in the horse.

The defining characteristics of OA are pain, stiffness, restriction of movement, catabolism of cartilage, and, in advanced cases, erosion of subchondral bone (Innes et al., 2010). Cartilage catabolism involves loss of matrix proteoglycans as breakdown exceeds neosynthesis. The roles of COX-2 in OA have not been fully elucidated, but the enzyme is up-regulated and PGE_2 concentrations in synovial fluid are increased in inflammatory joint diseases but not in DJD in the horse (May et al., 1992). Nevertheless, it is likely that PGE_2 is involved in the pain of OA and it also suppresses chondrocyte proliferation, increases IL1-β release (which is catabolic for cartilage), and inhibits chondrocyte aggrecan synthesis. It may therefore contribute to the loss of cartilage matrix.

Carprofen enhances SO_4 incorporation to stimulate the synthesis of proteoglycans by equine chondrocytes and cartilage explants in culture as well as reversing IL-1–induced suppression of proteoglycan synthesis by equine chondrocytes (Armstrong and Lees, 1999b, 2002; Frean et al., 1999). Carprofen exerts a similar action on canine chondrocytes (Benton et al., 1997). Whether these in vitro actions have significance for clinical subjects is not clear. However, a disease-modifying action of carprofen has been demonstrated in the dog in the Pond–Nuki model of OA (Pelletier et al., 2000).

Preclinical assessment of analgesic doses of NSAIDs for joint pain is often obtained using models providing intense but transient pain, for example, urate crystal–induced synovitis (Millis et al., 2002; Toutain et al., 2001b). While such models differ from clinical OA, it may be that they provide a major challenge to analgesic efficacy and are therefore justified for dosage selection for OA treatment. Toutain et al. (2001a,b) have carefully validated these models, comparing them (i) with data for COX inhibition in whole blood assays and (ii) against a Freund's adjuvant arthritis model in the dog.

Many controlled and randomized clinical trials have been undertaken to compare COXIBs with older drugs in dogs with OA for efficacy and tolerability. As regulatory authorities require, for licensing purposes, demonstration of noninferiority to a licensed drug, this has been the general outcome for efficacy (with occasional indication of superiority), whilst safety/tolerability profiles have been equal or superior to comparator products. Such findings have been shown for: firocoxib in comparison with carprofen or etodolac; mavacoxib in comparison with carprofen or meloxicam (Payne-Johnson et al., 2015; Walton et al., 2014); and robenacoxib compared to carprofen (Edamura et al., 2012; Reymond et al., 2012). Bennett et al. (2013) reported decreased lameness in OA dogs together with a reduction in synovial fluid C-reactive protein in response to robenacoxib. NSAIDs are used to treat some immunological diseases, such as systemic lupus and rheumatoid arthritis, where they may stimulate T-suppessor cells in their action against T-helper cells and autoantibody-producing B cells.

Antipyresis

NSAIDs lower body temperature in the presence of fever associated with microbial infections. The extent to which this is beneficial is unclear but human experience suggests improved feeling of well being. In animals, the antipyretic action of NSAIDs may encourage an earlier return to normal drinking and eating patterns and thereby aid recovery, when used concomitantly with antimicrobial drugs. Dipyrone is used only for its antipyretic properties but has been largely superseded by safer drugs; bone marrow toxicity is a potential concern with dipyrone.

Antihemostatic Actions of Aspirin

At clinically recommended dose rates, NSAIDs do not, as far as is known, markedly prolong bleeding times, although there are anecdotal reports that some prolongation occurs with some drugs. However, the action of aspirin on hemostatic mechanisms is unique in covalently acetylating COX-1, and inhibiting it irreversibly. Therefore, aspirin is the NSAID of choice as an antithrombotic drug, for example, in prophylaxis of aortic embolism in cats and in cats with hypertrophic cardiomyopathy to reduce the potential for thrombus formation and when a saddle thrombus of the aorta or iliac arteries has been diagnosed. In dogs with proteinuric glomerulonephritis, low-dose aspirin has been recommended for prevention of thromboembolic complications. The antithrombotic action of aspirin may also be of value in equine conditions, such as laminitis, navicular disease, and disseminated intravascular coagulation, although definitive studies are lacking.

Cancer

Epidemiological studies on human patients receiving long-term treatment with NSAIDs have indicated a potential beneficial effect against colon cancers. Overexpression of COX-2 in epithelial colon cells causes resistance to apoptosis and promotion of tumor growth by stimulating the production of growth factors and by angiogenesis; cancerous colon tissue has increased PGE_2 concentrations. A possible mechanism of the procancerous action of PGE_2 is transactivation of epidermal growth factor receptor, promoting growth of colonic polyps and cancers (Pai et al., 2002). Colon tumor cells (adenomas and adenocarcinomas) show increased COX-2 and decreased COX-1 expression at mRNA and protein levels compared with normal colon epithelial cells, and a therapeutic role for COX-2 inhibitors has been established in the prevention, treatment, and palliation of certain cancers. In dogs, PGE_2 concentrations were increased in osteosarcoma tissue compared to normal bone (Mohammed et al., 2001). Moreover, COX-2 upregulation occurs in colorectal tumors and polyps in the dog, canine transitional cell bladder carcinomas, and canine osteosarcomas (Mullins et al., 2004).

NSAIDs may reduce cell numbers in various cancers, and studies demonstrating that they directly induce apoptosis in neoplastic cells and inhibit angiogenesis provide possible mechanisms of action. Clinically, in canine prostatic carcinomas, carprofen and meloxicam increased median survival time and piroxicam, meloxicam, deracoxib, and firocoxib have been used in the treatment of transitional cell tumors in dogs; Knapp et al. (1992) reported a reduction in tumor size with piroxicam. In dogs with rectal polyps, piroxicam reduced polyp size and suppressed clinical signs (Knottenbelt et al., 2000). Pang et al. (2014a) identified antiproliferative and proapoptotic effects of mavacoxib on canine cancer cell lines and cancer stem cells in vitro. They also demonstrated a role for COX-2 in osteosarcoma initiation (Pang et al., 2014b). Wolfsberger et al. (2006) reported that meloxicam at low concentrations (1–10 μM) *increased* numbers of D-17 canine osteosarcoma cells in vitro after exposure for 3 days, whereas high concentrations (100 and 200 μM) exerted a marked antiproliferative effect. It should be noted that the maximum serum concentrations of meloxicam in the dog after therapeutic doses are 1.3 μM (oral dosing) and 2.1 μM (subcutaneous injection), and most of the drug is bound to protein (Busch et al., 1998).

In humans low-dose aspirin (81 mg daily), but not a higher dose (325 mg daily), reduced the adenomas occurring in colon cancer (Baron et al., 2003), but in another study the 325 mg dose of aspirin did suppress adenomas (Sandler et al., 2003). Neither the low nor the high dose is likely to produce significant and persistent COX-2 inhibition, whereas the antiplatelet COX-1 inhibitory actions are exerted at both the low and higher doses. Furthermore, much evidence correlates platelets to cancer spread. Nevertheless, low-dose aspirin (82 mg) does reduce colonic prostanoid synthesis by more than 50% and a higher daily dose (650 mg) produced no greater inhibition (Krishnan et al., 2001; Sample et al., 2002). Antiproliferative effects of COX inhibitors have been described in COX-negative cell lines, so that non-COX mechanisms of action are possible (Waskewich et al., 2002; Wick et al., 2002).

Mastitis, Metritis, and Endotoxemia

E. coli (acute and peracute) is a major welfare concern and source of economic mastitis loss in the dairy industry, involving reduced milk production, treatment costs, discarded milk, and mortality. As well as inflammation in the affected quarters, systemic signs include depression, fever, tachycardia, neutropenia, and inhibition of reticulorumen motility. Endotoxin causes the release or de novo synthesis of many inflammatory mediators and endotoxemia may be regarded as an acute inflammatory condition of the circulatory system. Endotoxic shock may be associated with a range of diseases in addition to mastitis, including equine colic, metritis, and septic peritonitis. Several reports in the 1980s indicated a therapeutic role for phenylbutazone and flunixin in equine endotoxemia (Moore, 1986; Moore et al., 1986) and for septic shock in dogs (Hardie et al., 1983). NSAIDs must be administered as soon as possible after onset of these conditions together with other supportive therapy.

For the treatment of acute mastitis and endotoxemia, systemically administered NSAIDs are used as adjuncts to antimicrobial drugs. For example, responses to carprofen therapy included lowering of rectal temperature, restoration of reticulorumen motility, and more rapid normalization of the clinical severity score in an *E. coli* model of mastitis (Vangroenweghe et al., 2005). These findings confirm the reports of previous workers for flunixin (Anderson et al., 1986a,b; Lohuis et al., 1989; Ziv and Longo, 1991), flurbiprofen (Lohuis et al., 1989; Vandeputte-Van Messom et al., 1987), indomethacin (Burvenich and Peeters, 1982), ketoprofen (Shpigel et al., 1994), phenylbutazone (Shpigel et al., 1996), dipyrone (Shpigel et al., 1996), and meloxicam (Banting et al., 2000). In addition to beneficial clinical responses, Anderson et al. (1986b) reported reduced PGE_2 and TxA_2 concentrations in the udder in response to flunixin; increased whey IgG1 and IgM concentrations were also obtained. Insofar as the effects of endotoxin are COX-mediated, it is likely that both isoforms are implicated. Therefore, a nonselective COX inhibitor may be the most rational therapeutic choice.

Systemically administered NSAIDs may be of less benefit in cases of mild or chronic mastitis. Pyorala et al. (1988) examined the influence of phenylbutazone and flunixin in cases of chronic subclinical mastitis. Neither drug influenced bacterial growth, somatic cell count, or inflammatory markers. However, Fitzpatrick (1998) reported that allodynia was abrogated by a single intravenous dose of flunixin. Further work is required to establish optimal dosage regimens for inflammatory conditions in cattle. Some studies have shown no beneficial effect of NSAIDs in reproductive disorders, such as retained placenta and postpartum endometritis (Konigsson et al., 2001). However, flunixin reduced the incidence of pyrexia in cows with either acute or subacute metritis (Amiridis et al., 2001). Uterine involution and onset of estrus occurred earlier in cows that received flunixin. It should be noted that the withdrawal time of flunixin should be increased from label recommendations when given to cows with severe clinical mastitis (Kissel et al., 2015).

Respiratory Diseases

Acute and potentially life-threatening inflammatory lung infections occur in calf and piglet pneumonias of viral, bacterial, or mixed etiology. Disease models and/or clinical trials have indicated positive responses to NSAID treatment; flunixin reduced lung consolidation scores in calves infected with P13 virus (Selman et al., 1984). However, other findings have been conflicting.

The effects of flunixin on lung lesions, postmortem lung weights (reflecting an antiedematous action) and clinical signs in an experimental bovine pasteurellosis model were investigated by Selman (1988). Benefits were apparent when used in combination with oxytetracycline. Anderson (1988) investigated a flunixin/oxytetracycline product in comparison with oxytetracycline alone in field cases of pneumonia. Reduction in coughing, return to normal food intake, and weight gain were better improved with the combination product and there were fewer relapses.

Other NSAIDs have been used as ancillary agents with antibiotics in the therapy of calf and/or piglet pneumonias. Not all studies have demonstrated clear benefits of NSAIDs, and the mechanism of any beneficial actions is not established. One may speculate that the antiinflammatory action may reduce pulmonary edema and improve lung function and respiratory gas exchange, while the antipyretic action may improve clinical status, so that animals begin to eat and drink. As cattle rely principally on respiration for temperature regulation, they may be stressed from infections causing pyrexia and hence benefit from the antipyretic actions of NSAIDs. The analgesic action of NSAIDs might also contribute to improvement in clinical status. As NSAIDs induce apoptosis, this may minimize release within the lungs of inflammatory mediators, which occurs when leukocytes are destroyed by viruses and bacteria. Carprofen, flunixin, ketoprofen, tolfenamic acid, and meloxicam possess marketing authorizations for use in the therapy of pneumonia in calves and/or piglets.

Ingestion of 3-methylindole in cattle causes a chemical toxicosis (fog fever) characterized by signs of respiratory distress, lung congestion, edema, and interstitial emphysema. Flunixin reduced respiratory rate and the extent of lung lesions, assessed by pathological and histopathological examinations, reducing the degree and severity of alveolar epithelial hyperplasia (Selman, 1988).

Calf and Piglet Scours

Infections of the GI in young calves and piglets are associated with high morbidity and, in the absence of effective treatment, possible mortality. Early studies suggested possible benefits from aspirin treatment. Jones et al. (1977) described the benefit of flunixin therapy in suppressing inflammation, reducing fluid losses in feces, and lowering morbidity and mortality in calf scours. Meloxicam is licensed for use in calf scours in combination with antimicrobial drugs.

Ophthalmic Uses

NSAIDs are used to treat conditions such as keratitis and scleritis by topical application; they do not inhibit reepithelialization of the cornea. Flunixin, phenylbutazone, and firocoxib have been used systemically in the management of nonulcerative keratouveitis and corneal ulceration in the horse. Aspirin and flunixin may be of value in intraocular surgery to minimize postoperative increase in aqueous humor protein concentration.

Neurodegenerative Diseases

COX-2 is up-regulated in the hippocampus and cortex in human subjects with dementia. PGs decrease neuronal function and accelerate neurodegeneration in patients with Alzheimer's disease (Hwang et al., 2002). Human epidemiological studies have indicated a lower incidence of Alzheimer's disease in subjects receiving a NSAID prior to the onset of neurological symptoms and on long-term NSAID therapy (Launer, 2003). This may be linked to their antiinflammatory properties; COX-2 containing inflammatory cells are present in the vicinity of amyloid-β plaques. However, there was no slowing of cognitive decline in patients with mild to moderate Alzheimer's disease receiving rofecoxib and naproxen over 12 months (Aisen et al., 2003). Stewart et al. (1997) reported relative risks of 0.4 and 0.65 for patients receiving NSAIDs for more than and less than 2 years, respectively. However, for acetaminophen the relative risk was 1.35. The mechanism of action of NSAIDs is unclear and does not necessarily involve COX inhibition.

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Section 5

Drugs Acting on the Cardiovascular System

21

Digitalis, Positive Inotropes, and Vasodilators

Clarke E. Atkins and Marisa K. Ames

An understanding of the pharmacokinetic and pharmacodynamic characteristics of cardiac drugs is essential for veterinarians who treat cardiovascular disease. Cardiac disease is often severe and life-threatening events must be managed with these drugs. Also, states of cardiac dysfunction that are amenable to drug therapy are common in dogs and cats. This chapter considers some of the more important drugs used in therapeutic management of cardiac disorders, with focus on those that affect basic aspects of cardiovascular function, including positive inotropes, inodilators, and vasodilators. Antiarrhythmic drugs are covered in Chapter 22. Other classes of drugs that elicit prominent cardiac responses (e.g., adrenergic and cholinergic agents) are discussed in Chapter 8, while off-loading therapies, other than vasodilators and inodilators (i.e., diuretics) are discussed in Chapter 24.

Basic Aspects of Cardiac Function

The primary pathways by which the cardiovascular system can increase or decrease cardiac output, based on need, include changes in heart rate, adjustments in myocardial contractility, an intrinsic response of the cardiac muscle to changes in muscle length, and optimization of vascular size (vasodilation and vasoconstriction). This physiological (neurohormonal) control is via pressure sensors, the central nervous system, sympathetic and parasympathetic nervous systems (SNS, PSNS), and the renin–angiotensin–aldosterone system (RAAS). The control systems involved are of considerable importance to pharmacology because the net response of the heart and vascular system is to these regulatory systems and the drugs administered often provide their effect via stimulating or blunting these systems.

Intrinsic Regulation

Contractile response of cardiac muscle to a change in its own length is the primary mechanism whereby the heart adjusts its pumping activity under normal physiological conditions (Fozzard, 1976). When venous return increases, the contractile function in the healthy heart increases, thereby pumping an increased volume of blood into the arterial system. This fundamental capability of the heart to autoregulate its pumping capacity in response to end-diastolic filling is referred to as the *Frank–Starling law of the heart* (Frank, 1895; Starling, 1918). This force–length relationship is primarily a result of an increase in calcium sensitivity as the initial sarcomere length increases. The relationship between preload (end-diastolic filling) and cardiac output under basal conditions and under dominance by the sympathetic and parasympathetic nervous systems is shown in Figure 21.1.

Regulation by the Nervous System

The autonomic nervous system regulates the cardiovascular system mainly by adjusting heart rate, vascular volume, and myocardial contractility. Details concerning cardiac effects and mechanisms of action of the sympathetic neurotransmitter norepinephrine (NE) and the parasympathetic neurotransmitter acetylcholine (ACh) are found in Chapters 6–8.

The intrinsic heart rate is determined via blockade of both arms of the autonomic nervous system. When an animal is at rest, the PSNS is likely dominant, as the resting heart rate is lower than the intrinsic heart rate. Patients with heart failure often have higher resting heart rates and less heart rate variability, suggesting that the SNS is likely dominant over the PSNS at rest. Sympathetic stimulation of cardiac muscle (via endogenous NE and certain pharmacological agents) can markedly increase the force of contraction, irrespective of end-diastolic muscle length. A change in contractile strength that is independent of muscle length is referred to as a change in contractility or inotropy. In the presence of inotropic stimulation by the sympathetic system, cardiac output at each level of ventricular filling is enhanced over the basal state (Figure 21.1). Conversely, parasympathetic nerves exert their primary influences

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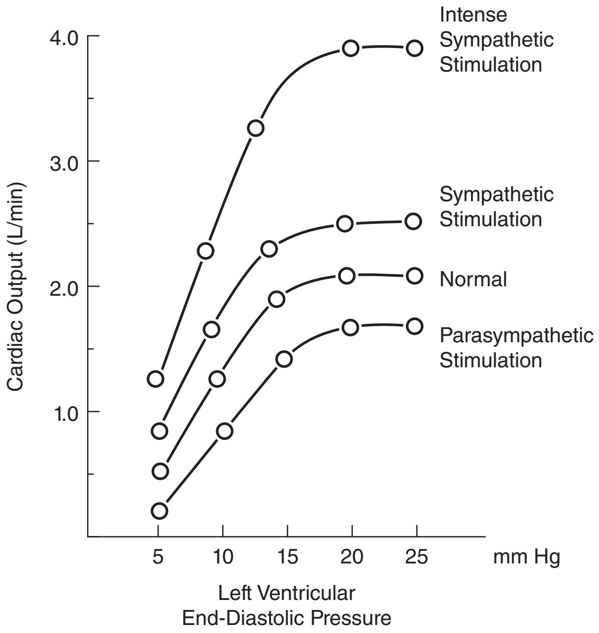

Figure 21.1 Frank–Starling law of the heart. As end-diastolic ventricular volume (preload) increases, the myofiber is stretched, enhancing the contractile state of the muscle; cardiac output is thus increased. The cardiac output curve can be influenced by different degrees of sympathetic and parasympathetic stimulation.

on cardiac output, not by changing the inotropic state, but by slowing heart rate and, with that, increasing ventricular filling time. Vagal discharge, however, when it produces bradycardia, decreases cardiac output at all levels of venous return and stretch on myocardial fibers (Figure 21.1). In contrast, sympathetic stimulation produces an increase in heart rate. Within physiological limits, cardiac output increases proportionately to the change in heart rate. Importantly, myocardial blood flow is decreased in rapid tachycardia, while myocardial oxygen needs are increased.

Myocardial oxygen demand (MVO_2) varies directly with three main factors: heart rate, myocardial wall tension, and inotropic state. Myocardial wall tension is directly related to ventricular radius (cardiac size) and intraventricular pressure and indirectly related to wall thickness (i.e., the law of Laplace). Primary determinants of ventricular wall tension are preload (i.e., end-diastolic volume and stretch) and afterload (determined by cardiac luminal size, wall thickness, and systemic blood pressure). By reducing pre- or afterload, certain drugs can elicit marked reduction in cardiac work (and MVO_2) without direct inotropic action on the heart muscle cell.

Cellular Concepts

The basic contractile unit of a heart muscle cell is the sarcomere, composed of actin (thin filament) and myosin (thick filament) proteins. A protein assembly unit, associated with the actin molecule, composed of tropomyosin and troponin, regulates activation of the filaments. Availability of ionized calcium (Ca^{++}) in the vicinity of troponin is the obligate modulator of the diastole–systole contraction cycle. Binding of Ca^{++} to a high-affinity subunit of the troponin molecule evokes the movement of tropomyosin from its diastolic blocking position on actin. Cross-linkages or “cross-bridges” are formed between projections of the myosin molecules and exposed sites on actin. As cross-bridges are formed, the thick and thin filaments slide over each other, and contraction occurs. Calcium delivery to the myofibrils is initiated by bioelectric events at the cell membrane, represented by the cardiac action potential.

Excitation–Contraction Coupling

L-type Ca^{++} channels are opened as the wave of depolarization travels down the T tubules, leading to the release of a small amount of Ca^{++}. This Ca^{++} triggers the activation of Ca^{++} release channels on the sarcoplasmic reticulum (SR) and causes the release of relatively large amounts of Ca^{++} into the cytosol (Opie, 2001; Fabiato and Fabiato, 1979).

Two separate pathways of movement of superficial Ca^{++} are believed to be involved (Langer, 1976, 1980; Parker and Adams, 1977). The primary electrogenic route is associated with the previously discussed triggered Ca^{++} release from the SR. An additional influx of Ca^{++} is linked with a Ca^{++}–Na^+ exchange across the sarcolemma. A schematic representation of excitation–contraction coupling in mammalian heart muscle is shown in Figure 21.2.

Relaxation

During repolarization, Ca^{++} is actively sequestered by the SR, which avidly binds and stores myoplasmic Ca^{++} with affinity greater than troponin. Relaxation occurs as Ca^{++} moves to the SR from troponin binding sites on the myofibrils, and the cytoplasmic Ca^{++} concentration decreases below the threshold required to trigger actin–myosin cross-bridge formation (Figure 21.2). Since this is an energy-requiring process, relaxation is somewhat a misnomer for the changes that occur during diastole.

Maintenance of Electrolyte Gradients

There is a net influx of Na^+ and Ca^{++} and efflux of K^+ with each action potential. Membrane-bound enzymes act as pumps to relocate ions and prevent their improper accumulation (Gadsby, 1984). Sodium–potassium–activated adenosine triphosphatase (Na^+,K^+-ATPase), localized in the cell membrane, propels Na^+ out of and K^+ into the cell, against their respective concentration gradients. Excess intracellular Ca^{++} is pumped out of the cell by systems localized in regions of the SR in close approximation

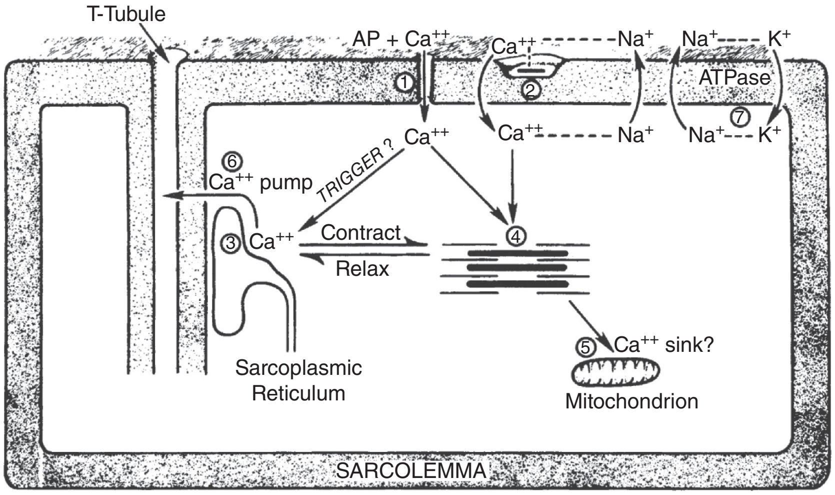

Figure 21.2 Schematic representation of cellular ion movements controlling excitation–contraction coupling in heart muscle. An action potential (AP) instigates the inward movement of Ca^{++} through slow Ca^{++} channels of the sarcolemma (1). Inward-moving calcium fills sarcoplasmic reticulum stores of the cation and also serves as a trigger to release additional Ca^{++} from storage sites of the sarcoplasmic reticulum (3). These Ca^{++} sources and that resulting from Na^+–Ca^{++} exchanges across the sarcolemma (2) activate the contractile proteins (4). Relaxation occurs as calcium is sequestered at storage sites of sarcoplasmic reticulum (3). Mitochondrion (5). Ca^{++} is pumped out of the cell (6). Altered sodium pump activity (7) may also affect sodium concentrations available from Na^+–Ca^{++} exchange. Source: Parker and Adams, 1977.

to the sarcolemma (Figure 21.2). A sarcolemmal Ca^{++}-ATPase also contributes to extrusion of Ca^{++}.

Positive Inotropes and Inodilators

Digitalis and Related Cardiac Glycosides

Digitalis and several closely allied chemicals are derived from the purple foxglove plant (*Digitalis purpurea*), other related species of the figwort family, and some plant species unrelated to digitalis.

Chemistry and Sources

Chemical and structure–activity relationships of the digitalis glycosides are quite complex, but several basic similarities are retained in the different compounds. The nomenclature is based on botanical origins rather than chemical structure. Digitalis is the dried leaf of the purple foxglove plant. Digitoxin, digoxin, and gitoxin also can be extracted from the leaf of a related plant, *D. lanata,* the woolly foxglove. Strophanthidin and ouabain are glycosides contained in the seeds of *Strophanthus* sp. Digitoxin and ouabain have been removed from the commercial market and only digoxin will be discussed here. Because of considerable pharmacological similarities between the different glycosides, the collective term *digitalis* has been used to designate the entire group of drugs, including digoxin. Often, *digitalis* and *digoxin* are used interchangeably by cardiologists. The term *glycoside* in general refers to a compound linked by an oxygen atom to a sugar molecule(s). The basic steroid-type nucleus is a cyclopentanoperhydrophenanthrene, to which is attached an unsaturated lactone ring at carbon atom 17 (C-17). The sugar molecules usually are attached at C-3; they influence water solubility, cell penetrability, duration of action, and other pharmacokinetic characteristics. The cardioactivity of the molecule resides principally in the aglycone moiety, but the positive myocardial actions of these entities are somewhat less potent and of lesser duration than the parent glycoside.

Cardiovascular Effects

Improved myocardial contractility was once considered to be the most important trait of the glycosides and, indeed, is the primary action on which the hemodynamic benefits depend. Today however, neuroendocrine (neurohormonal) effects, including reduced SNS activity and heart rate control, are believed to be of most important in treating heart failure.

Myocardial Contractility

The ability of cardiac glycosides to increase contractility has been demonstrated in a multitude of experimental preparations with results that validate a direct effect on contractile strength, independent of changes in resting fiber length, heart rate, or afterload. The positive inotropic action of cardiac glycosides is most pronounced in the hypodynamic or failing heart, though it is less than with other inotropic agents, such as dobutamine. Digitalis was shown in one study to provide a 24% increase in cardiac index versus 34% with dobutamine CRI (continuous rate infusion; ~3 μg/kg/min), meaning that the latter has a 42% greater inotropic effect than digoxin (Vatner et al., 1974).

Cellular Mechanisms of Inotropic Action

One of digitalis' proposed actions in congestive failure is a positive cardiac inotropic effect, but significance of this effect has diminished after examining results of several investigations. The second restorative action is neurohormonal normalization, thought by many to be the more important of known and postulated mechanisms of digitalis' clinical benefits (see Section Neuroendocrine Effects) (Ferguson, 1989).

Inhibition of Na$^+$,K$^+$-ATPase: The Mg^{++}-dependent Na$^+$,K$^+$-ATPase of the cell membrane supplies energy for the active pumping of Na$^+$ outward and K$^+$ inward against their large concentration gradients (Figure 21.2). The Na$^+$,K$^+$-ATPase is believed to be the cellular receptor for digitalis glycosides (Schwartz, 1977; Akera and Ng, 1991; Schatzmann, 1953). Inhibition of Na$^+$,K$^+$-ATPase by digitalis results in progressive reduction of $(K^+)_i$ as the ability of the pump to transport K$^+$ inward and Na$^+$ outward progressively fails (Fozzard and Sheets, 1985; Katz et al., 1985). A decrease in $(K^+)_i$ and/or an increase in $(K^+)_o$ reduces resting membrane potential to a less negative value, which can lead to increased automaticity and eventually impaired conduction and excitability. Inhibition of ATPase and resulting depletion of $(K^+)_i$ are responsible for the *arrhythmogenic properties* of digitalis.

The *inotropic effect* involves activation of a Na$^+$–Ca^{++} exchange mechanism through accumulation of $(Na^+)_i$. Baker et al. (1969) demonstrated with the giant squid axon that an increase in $(Na^+)_i$ enhanced the uptake of Ca^{++} by a Na$^+$–Ca^{++} exchange process. This mechanism seems to be operative in other excitable tissues and has been evoked as the link between inhibition of Na$^+$,K$^+$-ATPase and digitalis inotropy in the heart (Langer, 1977). The sequence of events can be visualized to include the following progression: digitalis interacts with and inhibits cell membrane Na$^+$,K$^+$-ATPase, outward pumping of Na$^+$ is slowed, $(Na^+)_i$ accumulates, increased $(Na^+)_i$ augments transmembrane exchange of intracellular Na$^+$ for extracellular Ca^{++}, $(Ca^{++})_i$ is increased, and Ca^{++} delivery to the contractile proteins is increased; thus the positive inotropic effect is gained. Dominance of Na$^+$–K$^+$ exchange and augmentation of Na$^+$–Ca^{++} exchange, with digitalis' inhibition of ATPase is demonstrated in Figure 21.3.

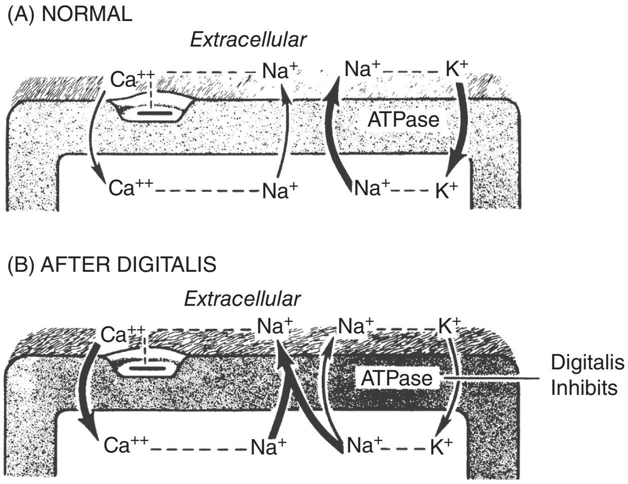

Figure 21.3 Schematic representation of a proposed mechanism for the positive inotropic action of cardiac glycosides. Inhibition of Na$^+$,K$^+$–ATPase (sodium pump) by digitalis results in increased intracellular concentrations of sodium available for exchange with calcium. Heavy arrows designate the dominant pathway of ion exchange during normal conditions (A) and after inhibition by digitalis of Na$^+$,K$^+$–ATPase (B). Adapted from Langer, 1976; Source: Parker and Adams, 1977.

Cardiac output

Digitalis glycosides increase contractility in both the normal and failing myocardium. However, the change in cardiac output is influenced by the functional status of the cardiovascular system.

Normal heart: Output of the normal heart increases minimally and may even decrease slightly after treatment with digitalis (Braunwald, 1985). Total peripheral resistance is increased by digitalis in the normal subject as a result of a centrally mediated increase in sympathetic vasomotor tone and direct vasoconstrictor effect. Impedance of the arterial circuit to ventricular ejection (afterload) is thereby increased, which attenuates the expected increase in cardiac output produced by the positive inotropic effect.

Failing heart: The work capacity of the failing ventricle at any given end-diastolic volume or pressure is inadequate to generate a normal stroke volume (Figure 21.4). The ejection fraction is diminished accordingly, which increases residual blood in the ventricle after systole (Moalic et al., 1993). If diastolic filling continues at a near normal rate, the ventricle will dilate to accommodate increased end-diastolic volume. With the administration of digitalis, the above processes are reversed. Digitalis-increased myocardial contractility augments work capacity of the ventricle, at any given end-diastolic filling pressure, as illustrated in Figure 21.4, where ventricular function curves derived in the prefailure state (normal) are compared with curves derived from congestive failure patients prior to and after digitalis therapy. Digitalis shifts the complete ventricular function curve upward in the direction of improved contractility (Mason, 1973; Braunwald, 1985). Systolic emptying is now more complete (increased ejection fraction) and, therefore, residual ventricular volume is diminished. Cardiac output increases and the size of the heart is reduced.

Digitalis' augmentation of myocardial contractility and normalization of baroreceptor function favorably affect vasomotor tone, evoking peripheral vasodilation with diminished outflow impedance (afterload). Additionally, improved cardiac performance increases venous return to the heart, thereby increasing preload and further enhancing cardiac performance by the Frank–Starling mechanism. This sequence of events continues to dominate as peripheral perfusion and tissue oxygenation improve, and it compensates for the direct vasoconstrictor effect of digitalis. The increase in cardiac output persists as long as the state of myocardial compensation prevails.

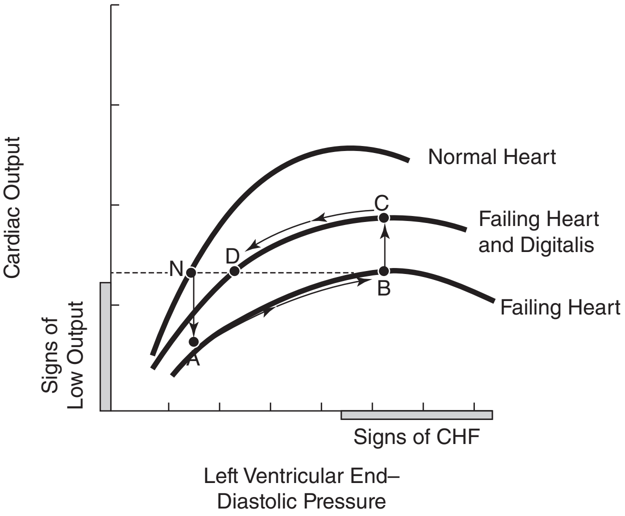

Figure 21.4 Diagrammatic representation of how changes in left ventricular filling influence cardiac output by the Frank–Starling mechanism in a normal heart and in a failing heart before and after digitalis. The points N to D represent in sequence: N–A, normal cardiac output falls to A because of initial contractile depression from congestive heart failure (CHF); A–B, shift to higher end-diastolic filling and thus higher cardiac output in accord with the Frank–Starling law; B–C, increase in contractility after digitalization; C–D, reduction in use of Frank–Starling compensation, which digitalis allows. N, B, and D: identical cardiac output on the vertical axis but achieved at different end-diastolic filling pressure on the horizontal axis. Levels of cardiac output and end-diastolic filling associated with signs of low output (e.g., fatigue) or CHF (e.g., dyspnea, edema) are represented by the dotted areas. Source: Adapted from Mason, 1973.

Cardiac Energy Metabolism

Digitalis increases oxygen consumption proportionately with increased contractile force in nonfailing cardiac muscle (Lee and Klaus, 1971); but patients with heart failure do not show an increase in the myocardial oxygen consumption because of the slowing of heart rate and the reversal of SNS-derived vasoconstriction.

These seemingly contradictory data can be reconciled by comparing the cardiodynamics of digitalis in normal and failing hearts. The heart with a normal ventricular volume responds to digitalis with increase in oxygen consumption commensurate with increase in contractility. Increased oxygen consumption is the direct result of increased contractility, in accordance with the concept that MVO_2 is influenced directly by the inotropic state, heart rate, and wall tension. Ventricular wall tension is directly proportional to ventricular pressure and radius (tension = (pressure × radius)/wall thickness; the Laplace relation). Tension will decrease if either pressure or radius is reduced. In the failing and dilated heart, reduction in cardiac size secondary to the inotropic action of digitalis therapy leads to a significant reduction in wall tension, which in turn leads to decreased MVO_2. The ultimate determinant of cardiac output is then a balance of the positive effect of increased preload on force of contraction and its negative effect on afterload, by reduction in cardiac chamber size (Laplace relation). Blood pressure normalization after cardiac glycoside therapy is secondary to cardiodynamic improvement in the congestive failure patient (Figure 21.4).

Neuroendocrine Effects

In heart failure patients, a lowering of heart rate accompanies the positive inotropic effect. This is apparently the result of a *neuroendocrine effect* and has been recognized as perhaps more important than the positive inotropic effects at therapeutic dosages. It also appears that the neuroendocrine effects are independent from the positive inotropic action and occur at lower serum digoxin levels (<1 ng/ml) (Ferguson, 1989). Neuroendocrine effects are achieved through digitalis' increase (normalization) in the baroreceptor reflex sensitivity that has been lost, presumably because of high levels of circulating aldosterone, during heart failure (Weber, 2001). Digitalis restores baroreceptor sensitivity and thereby decreases sympathetic tone in patients with heart failure (Quest and Gillis, 1971; McRitchie and Vatner, 1976; Zucker et al., 1980; Ferrari et al., 1981). The neuroendocrine effects also are attributed to direct pharmacological vagal stimulation (parasympathomimetic effect) (Thames et al., 1982). The neuroendocrine effects are responsible for a decrease in sinus rate, afterload, and speed of atrioventricular (AV) impulse conduction, thereby reducing cardiac work and MVO_2. Ahmed and Pitt have shown that the positive effects of digoxin are maintained and survival benefits enhanced at serum digoxin concentrations of 0.7–0.9 ng/ml, *but not higher* (Ahmed et al., 2006a,b; Ahmed et al., 2008), for both systolic and diastolic failure.

Excitability and Automaticity

As described earlier the increased excitability of digoxin on the heart is caused by ion fluxes and changes in K^+ conductance. Pacemaker cells are characterized by phase 4 spontaneous depolarization (normal automaticity), which moves diastolic potential to the threshold required for activation of phase 0, causing spontaneous depolarization (see Chapter 22). Therapeutic doses of cardiac glycosides increase vagal tone and decrease sympathetic tone, decreasing the slope of spontaneous diastolic depolarization of the sinoatrial pacemaker. This reduces the firing frequency of the sinoatrial node, and hence heart rate. After pretreatment with atropine, or with relatively high doses of digitalis, the nonvagal effects dominate, and an increase in automaticity is observed; this response is prevalent in the specialized conducting systems of atria and, especially, the ventricles. A typical transmembrane potential recording of a subsidiary pacemaker cell prior to and after digitalis is shown in Figure 21.5. This increased automaticity evoked by cardiac glycosides is due to an accelerated rate of spontaneous

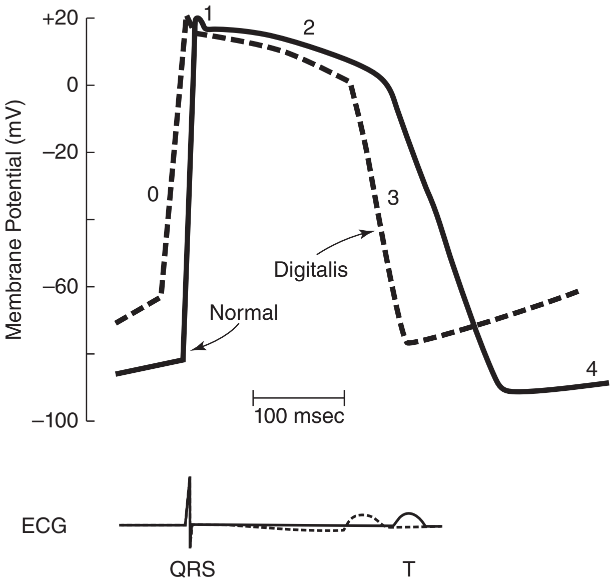

Figure 21.5 Electrophysiological effects of digitalis on transmembrane potential of a subsidiary pacemaker cell. Digitalis (i) decreases (less negative) the maximal diastolic potential, (ii) decreases the maximal rate of depolarization of phase 0, V_{max}, and (iii) enhances automaticity by increasing the slope of phase 4 spontaneous depolarization. (i) and (ii) lead to decreased conduction velocity and, in conjunction with (iii), can lead to arrhythmias of both impulse formation and impulse conduction. Source: Adapted from Mason et al., 1971.

diastolic depolarization. The normally latent pacemaker activities of cells within the ventricular conducting system are thereby magnified, leading to ectopic ventricular beats as an important early sign of digitalis toxicity. In contrast, muscle fibers in atria and ventricles can be depolarized to the point of inexcitability, without demonstrating spontaneous impulse generation. If excitability of ventricular muscle falls sufficiently, concomitant with increased frequency of ectopic impulses from specialized conduction fibers, the tendency for ventricular fibrillation is promoted.

The clinical significance of the “delayed afterdepolarizations” sometimes seen with digitalis toxicity is not completely resolved. These oscillations of the transmembrane potential initially are subthreshold and appear spontaneously during diastole after a usual action potential. These afterdepolarizations can, however, reach threshold as toxicity worsens, with the resulting extrasystoles contributing to ectopic arrhythmias associated with digitalis intoxication.

Impulse Conduction and Refractory Periods

The dominant effect of digitalis on impulse conduction is to slow conduction velocity by both vagal and nonvagal mechanisms. This response is particularly prevalent in the AV node and contributes importantly to the beneficial effects of digitalis in controlling ventricular rate during atrial fibrillation and flutter. Antiarrhythmic agents are discussed further in Chapter 22 of this book.

Digitalis Effects During Atrial Fibrillation and Flutter

During atrial fibrillation, the ventricular rate is rapid and dysrhythmic, as a result of rapid transmission of impulses through the AV node. This contributes further to cardiac dysfunction by promoting incomplete ventricular filling and ejection. Because digitalis prolongs the refractory period and delays impulse conduction through the AV node, fewer impulses will effectively reach the ventricle. Thus ventricular response rate is reduced to a slower, more physiological level (Ferguson et al., 1989).

Similar benefits are gained during atrial flutter. Digitalis can slow this rhythm or convert it to atrial fibrillation. Ventricular rate is still decreased, however, through prolonged AV refractoriness and slowed impulse conduction. Conversion of atrial flutter to fibrillation by digitalis is viewed optimistically because ventricular rate is controlled more easily during fibrillation than during flutter. It has been shown that the combination of digoxin and the Ca^{++} channel blocker, diltiazem (which also slows conduction through the AV node), is more effective at slowing the ventricular response to atrial fibrillation than is either drug alone (Gelzer et al., 2009).

Effects on the Electrocardiogram

The multiplicity of electrophysiological effects of cardiac glycosides on myocardial tissues can be expressed as equally complex changes in the electrocardiogram (ECG). Most conduction disturbances and dysrhythmias can be produced in normal individuals by administration of cardiac glycosides.

Congestive failure patients with sinus tachycardia or other supraventricular tachyarrhythmias usually demonstrate return toward more normal ECG patterns after digitalization. Rapid ventricular rates associated with atrial fibrillation or flutter are typically, but often inadequately, reduced by digitalis. Prolonged PR intervals, reflecting delayed AV conduction, are relatively common ECG features of digitalized dogs. Conversely, a lengthened PR interval is not necessarily a prerequisite for the therapeutic response.

Kidneys and Diuresis

After digitalization, reflex vasoconstriction decreases as cardiac output and hemodynamics are improved. Renal blood flow and glomerular filtration rate increase and stimuli for increased release of aldosterone are diminished. A fall in aldosterone secretion can be measured after digitalization in the dog with congestive failure (Figure 21.5). Diuresis results as salt and water retention by the kidneys is decreased. Diuresis, with lowering of capillary hydrostatic pressure, moves interstitial fluid back into the vascular space, providing relief from edema. Diuresis is not a prominent feature of digitalis therapy if edema does not accompany the congestive failure syndrome. Similarly, digitalis does not evoke diuresis if edema is not cardiogenic. Thus the diuretic response to digitalis is secondary to circulatory improvement and is not from a direct effect on the kidney, and is virtually never adequate without concurrent loop diuretic therapy (Robinson, 1972).

Pharmacokinetics

Dog: The absorption of digoxin after oral administration of an elixir usually is uniform, up to 75–90%, with peak serum concentrations attained in 45–60 minutes (Krasula et al., 1976). With tablets, the peak serum concentration is less, and occurs somewhat later (90 minutes). After IV administration, the maximal positive inotropic responses to digoxin were obtained within 60 minutes after injection (Hamlin et al., 1971). Breznock (1973, 1975) reported that the plasma half-life value for digoxin was 38.9 and 55.9 hours in different studies. Others report approximate digoxin half-lives varying from 24 to 31 hours, with a median of 30 hours for six studies (Barr et al., 1972; Doherty, 1973; Breznock, 1973, 1975; Hahn, 1977; DeRick et al., 1978). The importance of interpatient variability is exemplified in a study that found the half-life for digoxin in dogs at steady state varied from 14.4 to 46.5 hours (DeRick et al., 1978). These variables strengthen the need for adoption of individual dosage regimens, depending on the patient's response and, especially, evaluation of the serum digoxin concentration (SDC).

Digoxin is 25% protein bound (Breznock, 1973) and urinary excretion seems to be the most important route of elimination. Digitalis glycosides and their biotransformation products can follow an enterohepatic cycle, in which compounds are excreted by the liver into bile and some parent glycoside and metabolites are subsequently reabsorbed. See Section Digitalis in Cats.

Horse: The pharmacokinetics in horses have been studied because digoxin is occasionally considered for congestive heart failure and supraventricular arrhythmias in these animals (Sweeney et al., 1993). After oral administration of 44 μg/kg, the oral absorption was approximately 23%, with a peak concentration of 2.2 ng/ml (Brumbaugh et al., 1983). The half-life was approximately 17 hours based on mean serum concentrations. The authors presented oral doses needed to attain predicted steady-state serum concentrations that ranged from 28 μg/kg to 64 μg/kg loading dose, followed by 11–25 μg/kg maintenance dose every 12 hours. The specific dose depends on the therapeutic target concentrations cited in their paper (Brumbaugh et al., 1983).

Digitalis Toxicity

Plasma concentrations: In humans and dogs, therapeutic and toxic serum drug concentrations (SDCs) are in the range of 0.8–1.6 ng/ml and greater than 2.4 ng/ml, respectively (Moe and Farah, 1975). In dogs, concentrations from 0.8 to 2.4 ng/ml have been considered to be therapeutic, whereas SDC greater than 2.5–3 ng/ml are associated with increased probability of toxicosis. Nontoxic digoxin plasma concentrations have been determined for horses (0.5–2 ng/ml; Button et al., 1980); for cats (≤2.3 ng/ml; Erichsen et al., 1980); and for dogs (≤2.5 ng/ml; DeRick et al., 1978). Toxicity signs are generally mild or absent when serum digoxin concentrations are less than 2.5 ng/ml; moderate with SDC of 2.5–6 ng/ml; and severe, even fatal, when SDC exceeds 6 ng/ml (Fillmore and Detweiler, 1973; Teske et al., 1976).

Today, with the knowledge that neurohormonal and clinical benefits occur at much lower serum concentrations than needed for inotropic benefit (Ahmed et al., 2006a, 2006b; Ahmed et al., 2008) digoxin toxicity is much less a problem. In nonemergent situations, the authors advocate an initial low dosage, relying upon *other drugs discussed in this chapter* to control immediate signs. This is followed by uptitration of digoxin dosage over 2–3 weeks, using serum concentrations taken 8 hours posttreatment with a target SDC of 0.8–1.2 ng/ml. If kidney function declines, dose adjustments may be necessary to prevent accumulation of high concentrations of digoxin.

Clinical signs: Digitalis intoxication is characterized by clinical signs varying from mild gastrointestinal upset to neurological signs and death (Detweiler, 1977; Tilley, 1979). Relative inappetance, depression, and loose stools are common side effects, which are often self-limiting. Vomiting, however, is viewed more seriously, especially if protracted diarrhea is an accompaniment and these individuals should be examined for additional evidence of toxicity. Lethal outcomes are most often due to cardiac arrhythmias. The authors emphasize that the presence of any sign that can be seen with toxicosis should be considered to indicate toxicosis and action taken. This ideally triggers an office visit, SDC measurement, and serum biochemistry to evaluate kidney values. An ECG should be examined in dogs suspected of digoxin toxicity. The effects of digoxin on the ECG were described in Section Effects on the Electrocardiogram. Occurrence of ECG abnormalities necessitates: complete withdrawal of digitalis therapy; measurement of SDC; possibly other medical intervention; and reduction in dosage when reinstituted.

Electrolyte Involvement: Low K^+ concentration potentiates digitalis arrhythmogenicity and lessens efficacy of its treatment, whereas excess K^+ antagonizes arrhythmogenic activity. The antiarrhythmic activity of K^+ in digitalis intoxication is probably related to inhibition by the cation of glycoside binding to the Na^+,K^+-ATPase.

Treatment: Discontinuing digoxin administration is the first step to treat toxicity. Serum drug concentrations and ECG should be evaluated. Other drugs can be considered to replace digoxin in treatment (other drugs discussed in this chapter).

Digoxin immune FAB (Digibind®): This is an ovine source of antidigoxin antibodies. This treatment has been used in animals but is expensive. One vial contains 38 mg digoxin immune FAB and will neutralize 500 μg of digoxin. Animals will improve quickly after administration. After therapy with Digibind, free digoxin levels decreased whereas bound digoxin (to FAB) levels will rise.

Antiarrhythmic therapy: *Antiarrhythmic therapy for digitalis intoxication is not specific and treatment of specific arrhythmias is handled elsewhere* (see Chapter 22). Atropine may be helpful in cases with severe sinus bradycardia or AV block. In the presence of AV block, therapy with beta-blockers and calcium channel blockers should be avoided. Quinidine should be avoided as it may actually cause an increase in plasma concentrations of digoxin, probably by blocking the efflux transporter, p-glycoprotein (Discussed in Chapter 22).

Therapeutic Indications for Digitalis

Congestive heart failure: Controversy exists relative to the actual survival benefits of digitalis glycosides in the long-term therapeutic management of cardiac disease in animal and human patients (Hamlin et al., 1973; Patterson et al., 1973; Braunwald, 1985; Kittleson et al., 1985a). In man, no study has ever shown *overall* improvement in survival with digitalis therapy. However, improvement in quality of life and reduction in hospitalization have been demonstrated (Packer et al., 1993;Digitalis Investigation Group, 1997;Whitbeck et al., 2013). With low serum digoxin levels (0.5–0.9 ng/ml) there was a benefit, which was lost at higher serum levels (Ahmed et al., 2006a,b; Ahmed et al., 2008).

Cardiac glycosides are theoretically indicated in *systolic* heart failure of any etiology. New inodilator drugs (pimobendan discussed in Section Inodilators: Pimobendan) have largely taken the place of digitalis in managing heart failure in animals.

Atrial arrhythmias: Many specialists use digoxin (often with diltiazem) to control the heart rate in atrial fibrillation, when there is a rapid ventricular response (Gelzeret al., 2009).This therapeutic choice is made easier if heart failure is also present, especially with evidence of poor systolic function (e.g., dilated cardiomyopathy). However, digoxin does not produce conversion to a sinus rhythm, but reduces ventricular rate by slowing AV conduction (Meijler, 1985).

Precautions: The authors do not initiate digoxin treatment unless rate control is needed and/or if inotropic support is needed and finances precluded the use of other positive inotropic agents. Digitalis therapy is not used in the presence of heart block or ventricular tachycardia.

Digoxin should not be used to reduce heart rate when sinus tachycardia is present without evidence of heart failure. Tachycardia, associated with other conditions, such as fever, thyrotoxicosis, pain, anxiety, or even cardiac conditions, such as pericardial effusion with tamponade or with hypertrophic cardiomyopathy (HCM), is not amenable to digitalis therapy.

Clinical Practice

Generally, digoxin is initiated at the low end of the dose range listed below, then increased gradually if necessary to achieve the desired therapeutic outcome. If toxicity is observed, monitoring and assessment – as described in this chapter – should be used to adjust the dosage or make a determination about discontinuing digoxin administration. A listing of average digitalis glycoside dosages is provided in formularies for dogs and cats (Tables 21.1 and 21.2). The dosage regimens published for dogs have used both a mg/kg and mg/m^2 dosing schedule, the latter based on body surface area. There is some evidence that dosing on a body surface area basis may be safer than mg/kg dosing (Kittleson, 1983).

Parenteral schedules: IV administration increases the likelihood for toxicity, including life-threatening arrhythmias, and this route is rarely used for administration. Digoxin is sufficiently absorbed by the oral route that IV administration is not ordinarily necessary. Intravenous dosing regimens are provided in previous editions of this book.

Digitalis in Cats

There are less data on administration of digoxin to cats compared to dogs. There are no studies of survival or quality of life (Atkins et al., 1988, 1989, 1990); however, there are evaluated clinically relevant aspects of digitalis therapy in cats.

The effect of concurrent therapies on digoxin pharmacokinetics was measured and results presented in Table 21.3. The study showed that other drugs can affect digoxin through unidentified mechanisms. The authors also compared results between the 10 and 20-day evaluations, indicating that at steady state there is no effect

Table 21.1 Medical management of factors contributing to signs of systolic heart failure in dogs. Source: Adapted from Atkins CE. Atrioventricular valvular insufficiency. In Allen DG (ed): *Small Animal Medicine,* Lippincott, 1992.

Factor	Strategy	Agent and dosage
Fluid retention/ excessive preload	Salt restriction	Senior diet, renal diet or, late in course, heart (heavily salt-restricted) diet
	Diuresis	Furosemide 1–4 mg/kg SID–TID, IV, IM, SC, or PO or CRI at 0.66 mg/kg/min
		Torsemide 0.2 mg/kg PO SID–TID
		Hydrochlorothiazide or aldactazide 2–4 mg/kg QID–BID PO
		Chlorthiazide 20–40 mg/kg BID PO
		Spironolactone 2.0 mg/kg SID PO
	Venodilation	Triamterene 2–4 mg/kg/day PO
		Nitroglycerin 2% ointment 0.5 cm per 5kg TID topically for 1st 24 hours
		Captopril 0.5–2 mg/kg TID PO
		Enalapril 0.5 mg/kg SID–BID PO
		Benazepril 0.25–0.5 mg/kg SID PO
		Prazosin 1 mg TID if <15 kg; 2 mg TID if >15 kg
		Sodium nitroprusside 1–5 µg/kg/min IV
Neurohormonal aberration	Blunt RAAS	Captopril 0.5–2 mg/kg TID PO
		Enalapril 0.5–1 mg/kg SID–BID PO
		Benazepril 0.25–0.5 mg/kg SID PO
		Spironolactone 2.0 mg/kg/day PO
		Angiotensin II receptor blocker (e.g., losartan) dosage TBD
	Blunt SNS	Digoxin 0.005–0.01 mg/kg or 0.22 mg/m^2 body surface BID PO for maintenance
		Propranolol 5–40 mg TID PO
		Atenolol 0.25–1 mg/kg PO[b]
		Carvedilol 0.1–0.2 mg/kg SID PO, increasing to 0.5–1mg/kg BID over 6 weeks
Increased afterload	Arterial vasodilation	Hydralazine 1–3 mg/kg BID PO
		Captopril 0.5–2 mg/kg TID PO
		Enalapril 0.5 mg/kg SID–BID PO
		Benazepril 0.25–0.5 mg/kg SID PO
		Prazosin 1 mg TID PO if <15 kg; 2 mg TID if >15 kg PO
		Sodium nitroprusside 1–5 µg/kg/min IV
		Diltiazem 0.1–0.2 mg/kg IV slowly; 0.5–1.5 mg/kg TID PO
		Amlodipine 0.1–0.2 mg/kg SID–BID PO
		Sildenafil 0.5–1 mg/kg SID–BID PO
Diminished contractility[a]	Positive inotropic support	Digoxin 0.005–0.01 mg/kg or 0.22 mg/m^2 body surface BID PO for maintenance
		Rapid oral: 0.01 mg/kg BID to 0.02 mg/kg TID for 1 day, then to maintenance
		Rapid IV: 001–0.02 mg/kg given one half IV immediately and one-fourth IV at 30 to 60-min intervals PRN
		Dobutamine 1.5–20 µg/kg/min IV for <72 hours
		Dopamine 2–10 µg/kg/min IV for <72 hours
		Amrinone 1–3 mg/kg IV followed by 10–100 µg/kg/min
		Pimobendan 0.25 mg/kg BID PO

[a] In most instances of mitral insufficiency, positive inotropic support is unnecessary.

[b] Calcium channel (verapamil and diltiazem) and beta-blockers (propranolol, esmolol, atenolol) should be used with caution in patients in heart failure.

SID, once daily; BID, twice daily; TID, three times daily; QID, four times daily; IM, intramuscularly; IV, intravenously; SC, subcutaneously; PO, per os; PRN, as needed; RAAS, renin–angiotensin–aldosterone system; SNS, sympathetic nervous systems.

of duration of therapy on pharmacokinetics in normal cats (Atkins et al., 1988). Pharmacokinetics were also examined in cats with compensated dilated cardiomyopathy (DCM) compared with six clinically normal cats (Atkins et al., 1989) at a dosage of 0.01 mg/kg, q 48 h for 10 days. There were no differences between control and heart failure cats. Other studies have shown hemodynamic improvement in cats with heart failure due to DCM (Atkins et al., 1990).

One can conclude from these studies, and others, that digoxin is effective in cats with DCM, when given q 48 h, that concurrent treatment alters pharmacokinetics, predisposing to toxicity, but that the heart failure state per se and prolonged therapy does not further affect these parameters. The recommended dosage for cats in heart failure is 0.007 mg/kg q 48 h and SDC should be determined at steady state, 8 hours posttreatment, with a goal of 0.8–1.2 ng/ml.

Table 21.2 Feline formulary

Drug	Trade Name[a]	Formulation(s)[b]	Dosage	Use
Amlodipine	Norvasc	1.25 mg tablets	0.625 mg PO QD–BID	Antihypertensive
Diltiazem	Cardizem	30 mg tablets	7.5 mg PO TID	Lusitrope, Vasodilator, Negative chronotrope
Diltiazem - LA				
	Dilacor XR	180, 240 mg capsule	30 mg PO BID	*same*
	Cardizem CD	180, 240 mg capsule	45 mg PO QD	*same*
Enalapril	Enacard (Vasotec)	1, 2.5, 5 mg tablet	0.5 mg/kg PO QD	ACEI (CHF, Hypertension)
Benazepril	Lotensin (Foretkor)	5, 10 mg tablet	0.25–0.5 mg/kg PO QD–BID	*same*
Atenolol	Tenormin	25 mg tablet	6.25–12.5 mg PO QD	Negative chronotrope, Antiarrhythmic, Lusitrope, Antihypertensive
Esmolol	Brevibloc	10, 250 mg/ml injectable	50–500 (100 usually) µg/kg IV	*same*
Sotalol	Betapace	80 mg tablet	2 mg/kg PO BID	Antiarrhythmic
Procainamide	Pronestyl, Procan SR	250 mg tablet100 mg/ml injectable	2–5 mg/kg PO BID–TID	Antiarrhythmic
Furosemide	Lasix	12.5 mg tablet 50 mg/ml injectable	1–4 mg/kg PO BID–q 48 h; 0.5–2 mg/kg SQ, IM, IV PRN	Diuretic
Nitroglycerin	Nitrol, Nitro-Bid	2% ointment	2–5 cm topically TID for 24 h	Venodilator (CHF)
Warfarin	Coumadin	1, 2, 2.5, 4 mg tablet	0.1–0.2 mg QD	Anticoagulant
Heparin		Multiple	250–300 U/kg SQ TID	Anticoagulant
LMW Heparin	Fragmin	2500 U/0.2 ml	100 U/kg SQ QD	Anticoagulant
Aspirin	Plavix	81 mg	40–80 mg q 72 h	Anticoagulant
Clopidogrel		75 mg	17.5 mg daily	Anticoagulant
Digoxin	Lanoxin	0.05 mg/ml elixir 0.125 mg tablet	0.007 mg/kg PO q 48 h (check serum [digoxin])	Positive inotrope, Negative chronotrope (CHF, SVT)
Taurine		250 mg tablet	250 mg PO QD	Taurine deficiency
Cyproheptadine	Periactin	4 mg tablet	2 mg BID	Prevent SAE vasoconstriction (?)

[a] Selected name brands, some available as generic.

[b] Most appropriate formulations for cats; other sizes available for many drugs.

BID, twice daily; TID, three times daily; IM, intramuscularly; IV, intravenously; SQ, subcutaneously; PO, per os; PRN, as needed.

Table 21.3 Digoxin pharmacokinetic properties in cats after treatment with digoxin tablets (0.01 mg/kg of body weight, q 48 h). Source: Adapted from Atkins et al., 1988.

Variable	Group 1 (n = 6)		Group 2 (n = 3)	
	DXN alone	DXN, FRS, ASA	DXN 10 days	DXN 20 days
Peak [DXN] (ng/ml)	2.1 ± 0.35 (0.95 to 3.69)	3.3* ± 0.60 (1.31 to 5.64)	1.8 ± 0.38 (1.11 to 2.69)	1.4 ± 0.08 (1.31 to 1.63)
8-Hour [DXN] (ng/ml)	1.4 ± 0.35 (0.56 to 3.03)	2.5 ± 0.64 (0.63 to 5.01)	1.1 ± 0.33 (0.58 to 1.91)	0.8 ± 0.29 (0.58 to 1.71)
Mean [DXN] (ng/ml)	1.1 ± 0.22 (0.44 to 1.85)	2.2* ± 0.57 (0.55 to 4.15)	0.93 ± 0.2 (0.57 to 1.41)	0.69 ± 0.1 (0.54 to 0.95)
t (hours)	40.1 ± 11.7 (13.2 to 99)	81.8* ± 21.8 (30.1 to 173)	61.8 ± 24.0 (23 to 119.6)	47.7 ± 13.8 (24.7 to 80.7)
Oral clearance (L/h.kg)	0.15 ± 0.035 (0.05 to 0.27)	0.07* ± 0.02 (0.01 to 0.17)	0.10 ± 0.02 (0.08 to 0.14)	0.16 ± 0.04 (0.07 to 0.24)
Hours [DXN] in toxic range	3 ± 1.7 (0 to 6)	24.7* ± 9.8 (0 to 48)	2 ± 1.6 (0 to 6)	0 ± 0 (0)

*Significantly different from that value within the same group (P <0.05).

ASA, aspirin; DXN, digoxin; FRS, furosemide.

Preparations

Digoxin, USP – cardiotonic glycoside from *D. lanata.*

Digoxin Injection, USP – digoxin in 10% alcohol; injections, 0.5 mg/2 ml.

Digoxin Tablets, USP – tablets, 0.25 and 0.5 mg.

Digoxin Solution – digoxin, 0.05 mg/ml in oral solution.

Sympathomimetic Agents: Dobutamine and Dopamine

Sympathomimetic drugs, such as dobutamine and dopamine, can be used to support cardiac function and blood pressure acutely. Veterinary cardiologists have most often employed dobutamine for the emergency management of heart failure in dogs; there is less support for administration of dopamine.

Dobutamine

This agent, a synthetic sympathomimetic, produces improvement in cardiac performance by complexing primarily with myocardial β_1 receptors, which, through second messengers, increase intracellular calcium and, thereby, myocardial contractility. There is both agonist and antagonist effects on α receptors, the clinical effects of which are uncertain. Dobutamine is unique as a SNS-agonist as it has relatively little effect on heart rate, is minimally proarrhythmic, and has a very short half-life (1–2 minutes). The short half-life allows for quick adjustment of dose rates and if the infusion is discontinued the effects quickly dissipate. The short half-life also requires that it be administered as a constant rate infusion (CRI). Additionally, it results in down-regulation of β_1 receptors 48–72 hours after its institution, rendering the drug ineffective after this time. Furthermore, there is concern that the positive dromotropic effect of dobutamine in dogs with atrial fibrillation, not receiving digitalis, may increase AV nodal conduction to a degree that a life-threatening ventricular response rate may result in ventricular fibrillation. There are no clinical trials involving dobutamine in natural canine heart disease, but anecdotal evidence that it may be helpful for the acute management of heart failure in hospitalized patients.

Dobutamine is beneficial for emergency management of DCM, without atrial fibrillation or other supraventricular tachycardia. It provides inotropic support without increasing heart rate and can be life-saving when there is profound myocardial systolic dysfunction. There is no evidence of benefit for treatment of long-standing mitral valve regurgitation.

Dopamine

Dopamine, unlike dobutamine, is not a synthetic catecholamine, occurring naturally and formed endogenously from L-Dopa. It has a significant first pass effect and very short half-life, dictating that it can only be administered IV with a CRI. It has two known receptor subtypes with which it interacts (DA_1 and DA_2). DA_1 subserves vasodilation in the renal, cerebral, mesenteric, and coronary vasculature. DA_2 stimulation inhibits norepinephrine release from the postsynaptic nerves and autonomic ganglia. However, dopamine also stimulates β_1- and $\alpha_{1,2}$-adrenoreceptors, with the expected positive inotropic, chronotropic, dromotropic, and vasoconstrictive effects.

With this wide spectrum of effects, it is not surprising that clinical effects vary with dosage. At a low dosage (0.5–1 μg/kg/min), dopamine stimulates DA_1 only, thereby lowering blood pressure, without other hemodynamic effects. At intermediate dosages, cardiac benefits are recognized, with variable effects on heart rate, a lack of reduction in pulmonary capillary wedge pressure, but positive effects on cardiac output and renal blood flow. At high doses, vasoconstriction may be observed with increased cardiac afterload. Dopamine does not dilate capacitance vascular beds, so is sometimes accompanied by venodilators (nitroglycerin or nitroprusside) or by dobutamine. Concerns at this dosage range include arrhythmias and tachycardia. High dosages (2–10 μg/kg/min in normal humans to >50 μg/kg/min in shock patients) produce, in addition to tachycardia and arrhythmia, elevation of blood pressure and systemic vascular resistance through α_1 and α_2-adrenoreceptor stimulation with resultant vasoconstriction (Horowitz et al., 1962; Sprung et al., 1984). This is desirable only in shock management and should be preceded by fluid therapy to correct deficits.

In veterinary medicine, dopamine has found its greatest utility in the management of hypotension during anesthesia and treating noncardiogenic shock. Sisson and Kittleson (1999) recommend a starting dosage of 2 μg/kg/min, with upward titration or retreat as the clinical situation dictates. The ultimate dosage typically ranges from 1 to 8 μg/kg/min, administered by CRI (in 5% D/W, using an infusion pump to avoid excessive volumes in heart failure patients). Although it has been used, at low doses, to stimulate DA receptors, dilate renal vessels, and treat acute kidney disease, it has not been shown to produce these benefits in animal studies.

Preparations: Supplied in 5-ml vials containing 40, 80, 160 mg/ml.

Inodilators: Pimobendan

Agents that have both vasodilatory and positive inotropic properties are classified as *inodilators* (Opie, 2001). Historically, short-term management of acute or decompensated heart failure characterized by systolic dysfunction benefited from a combination of dobutamine (positive inotrope) and nitroprusside (vasodilator). Inodilators combine these properties and agents such as pimobendan, which is available in an oral formulation, make chronic therapy a possibility. Levosimendan, another drug from this class having been investigated in animals, is not available for clinical use at this time.

Clinical Application

Pimobendan is a novel agent with properties useful in the clinical management of canine heart failure secondary to either DCM or myxomatous mitral valvular disease (MMVD). The efficacy of pimobendan in the treatment of heart failure, arising from DCM and MMVD, has been evaluated more thoroughly in dogs than have other cardioactive medications to date.

In dogs (English Cocker spaniels and Doberman Pinschers) with DCM and heart failure pimobendan was associated with a significant improvement in heart disease class (modified New York Heart Association [NYHA] functional class; overall, median NYHA 2 to NYHA 3), regardless of breed (Figure 21.6, Table 21.4). Other agents (furosemide, enalapril, and digoxin) also were allowed. However, only in the Doberman pinschers was there a significant survival benefit (Fuentes, 2004).

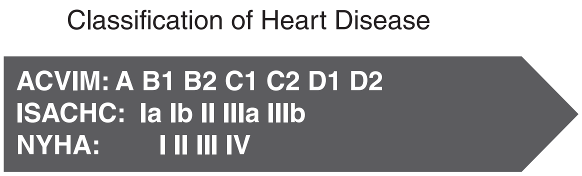
Classification of Heart Disease
ACVIM: A B1 B2 C1 C2 D1 D2
ISACHC: Ia Ib II IIIa IIIb
NYHA: I II III IV

Figure 21.6 The American College of Veterinary Internal Medicine Cardiac Disease Classification Scheme (ACVIM), the International Small Animal Cardiac Health Council (ISACHC) and New York Heart Association adaptation are categorically compared. See Table 21.4 for description. Source: Atkins et al. 2009.

Several high-quality studies have been performed on dogs with heart failure caused by cardiomyopathy or mitral valve disease (Lombard et al., 2006; O'Grady et al., 2008; Häggström et al., 2008; Summerfield et al., 2012). The results of these studies showed that pimobendan was effective, increased survival, and had a favorable safety profile. Some studies were multiinstitution, high-quality studies, for example, the QUEST trial (Häggström et al., 2008), the PROTECT study (Summerfield et al.,

Table 21.4 Functional classification schemes of cardiac disease in dogs

A. Modified New York Heart Association (NYHA) system (American)	
Class I	Includes patients with asymptomatic heart disease (typically murmur only)
Class II	Includes patients with signs of cardiac dysfunction only with strenuous exercise
Class III	Includes patients with heart disease that causes clinical signs with routine daily activities or mild exercise
Class IV	Includes patients with heart disease that causes severe clinical signs even at rest
B. International Small Cardiac Health Council (ISACHC) system	
Class I	The asymptomatic patient: Class IA: Signs of heart disease are present, but no signs of compensation (volume or pressure load ventricular hypertrophy) are evident Class IB: Signs of heart disease are present and signs of compensation (volume or pressure overload ventricular hypertrophy) are detected radiographically or echocardiographically
Class II	Mild-to- moderate heart failure; clinical signs of heart failure are evident at rest or with mild exercise, and adversely affect the quality of life
Class III	Advanced heart failure; clinical signs of advanced heart failure are immediately obvious: Class IIIA Home care is possible Class IIIB Hospitalization is recommended (cardiogenic shock, life-threatening pulmonary edema, or a large pleural effusion
C. American College of Veterinary Internal Medicine (ACVIM) scheme (adapted from American Heart Association and American College of Cardiology)	
Class A	Includes asymptomatic patients with no sign of cardiac disease but believed to be at risk because of genetic, environmental, or infectious causes
Class B1	Includes asymptomatic patients with heart disease, typically identified by the finding of a heart murmur, but without evidence of cardiac enlargement
Class B2	Includes asymptomatic patients with heart disease, typically identified by the finding of a heart murmur, which have evidence of hemodynamically significant valve regurgitation, evident by the finding of cardiac enlargement
Class C1	Includes patients with past or current clinical signs of heart failure, associated with structural heart disease, and requiring hospitalization
Class C2	Includes patients with past or current clinical signs of heart failure, associated with structural heart disease, which can be released from the hospital or which do not require hospitalization
Class D1	Includes patients in heart failure due to end-stage cardiac disease, which are refractory to standard therapy (requiring advanced or specialized treatment), requiring hospitalization
Class D2	Includes patients in heart failure due to end-stage cardiac disease, which are refractory to standard therapy (requiring advanced or specialized treatment) but that can be managed as outpatients

2012), and the EPIC trial. The EPIC study is a prospective trial, evaluating 360 client-owned dogs. EPIC, standing for evaluation of pimobendan in dogs with cardiomegaly caused by preclinical mitral valve disease, is a double-blind, randomized, placebo-controlled clinical trial, evaluating the effectiveness of pimobendan in the prevention of the onset of signs of congestive heart failure in dogs with cardiac enlargement secondary to preclinical myxomatous mitral valve disease. Preliminary results indicate that pimobendan is clearly beneficial and did not raise any concern over the administration of pimobendan.

In randomized prospective clinical trials, pimobendan has been proven to be safe and effective in heart failure secondary to both DCM (Doberman pinschers) and MMVD, as well as in asymptomatic DCM (Doberman pinschers) and MMVD. Pimobendan has been approved for use in dogs with congestive heart failure, beginning in 2000 in many countries around the world.

Pimobendan in cats: There are only a few studies to define the clinical use in cat. It produced improved survival in cats with nontaurine responsive dilated cardiomyopathy without adverse effects (Hambrook and Bennett, 2012). It improved survival and was well-tolerated in cats with congestive heart failure caused by cardiomyopathy (MacGregor et al., 2011; Reina-Doreste et al., 2014; Gordon et al., 2012). The pharmacokinetics have been performed in cats (Hanzlicek et al., 2012). The pharmacokinetic studies showed a rapid oral absorption and elimination half-life of 1.3 hours (mean). In other animals, pimobendan is metabolized (demethylated) to desmethylpimobendan, which is active and responsible for some of the cardiovascular effects. An observation from the feline pharmacokinetic study is that some cats may not be capable of metabolizing the parent drug to an active metabolite. The implications of the differences in metabolism among cats is undetermined until additional study results are available. The dosage is the same as in the dog, 0.25–0.3 mg/kg, q 12 h, oral. Administration of large oral tablets designed for dogs can be a challenge in cats.

Mechanism of Action

Inotropy: Pimobendan is a benzimidazole pyridazinone derivative that has effects as a positive inotrope and balanced arteriovenous dilator. In failing hearts it exerts its positive inotropic effects primarily through sensitization of the cardiac contractile apparatus to intracellular calcium. As a phosphodiesterase (PDE) III inhibitor, it can potentially increase intracellular calcium concentration and increase myocardial oxygen consumption. However, the cardiac PDE effects of pimobendan are reportedly minimal at pharmacological doses in dogs with heart disease, which is a major advantage relative to other inotropic PDE inhibitors such as milrinone. Pimobendan's calcium sensitization of the contractile apparatus is achieved by enhancement of the interaction between calcium and troponin C complex resulting in a positive inotropic effect that enhances systolic function, but does not increase myocardial oxygen consumption. The major advantage of pimobendan is its relative lack of the arrhythmogenic activity that is associated with positive inotropes whose sole mechanism of action is to increase myocardial intracellular calcium or cyclic AMP concentrations.

Vasodilation: Phosphodiesterase III and V are found in vascular smooth muscle. Inhibitors of PDE III, such as pimobendan, result in balanced vasodilation (combination of venous and arterial dilation) leading to a reduction of both cardiac preload and afterload, a cornerstone of therapy in heart failure. In addition, pimobendan may have some PDE V inhibitory effects. PDE V concentrations are relatively high in the vascular smooth muscle of pulmonary arteries; therefore, PDE V inhibition may ameliorate elevations in pulmonary artery pressure (pulmonary hypertension) that tend to parallel long-standing elevations in left atrial pressure, a clinically important complication of CVD. (Sildenafil is a specific PDE-V inhibitor, discussed in Section Phosphodiesterase V Inhibitors: Sildenafil.)

Cytokine modulation: The significance of alterations in proinflammatory cytokines, such as tumor necrosis factor-α and interleukins 1-b and 6, on the progression of heart failure has been documented in several forms of heart disease. Maladaptive alterations in these cytokine concentrations are associated with increased morbidity and mortality; pimobendan has demonstrated beneficial modulation of several such cytokines in models of heart failure (Iwasaki et al., 1999). Anecdotally, many clinicians feel that this results in attitude and appetite improvement beyond what is seen with control of heart failure alone.

Antiplatelet effects: In the dog, pimobendan has in vitro antithrombotic effects, but only at drug concentrations much higher than what can be clinically attained. Therefore, pimobendan's antiplatelet effects in the dog neither contributes to its therapeutic benefit nor confers risk of bleeding (Shipley et al., 2013).

Positive lusitropic effects: Via PDE III inhibition in cardiomyocytes, pimobendan increased intracellular cAMP, facilitating phosphorylation of receptors on the sarcoplasmic reticulum. Diastolic reuptake of calcium is thus enhanced, and the speed of relaxation increased, indicating a positive lusitropic property. Two small studies in the human and dog support this positive lusitropic property of pimobendan (Asanoi et al., 1994; Ishiki et al., 2000).

Pharmacokinetics

Pimobendan's absorption is rapid, with peak plasma levels achieved within an hour of oral administration. Thus, although pimobendan is an oral preparation, it can provide rapid, short-term support to dogs with emergent, acute-onset or decompensated heart failure. The elimination half-life in dogs is approximately 1 hour or less (Bell et al., 2015; Yata et al., 2016). Oral bioavailability is 60–65%, and as high as 70% (Bell et al., 2015), but because it is reduced in the presence of food, pimobendan should be administered at least 1 hour after feeding until steady state is reached. Pimobendan is water insoluble, highly protein bound (90%–95%), excreted into bile, and eliminated in the feces. Pimobendan is metabolized (demethylated) in the liver and the major metabolite desmethylpimobendan (UDCG-212) is a more potent inhibitor of PDE III (vasodilation) than pimobendan and has a half-life slightly longer than the parent drug (Yata et al., 2016) (as discussed in Section Pimobendan in Cats, cats may not produce this metabolite).

Adverse Effects

Pimobendan is well tolerated in dogs, including in animals with heart disease (Fuentes, 2004). There were initial concerns that it could produce worsening of (fatal) arrhythmias, or increase risk of atrial fibrillation in dogs with cardiomyopathy. In subsequent studies (Lake-Bakaar et al., 2015) these concerns were dismissed and no prospective, randomized, blinded trial has reported an increase in the frequency of arrhythmias in veterinary patients. Veterinary studies report improved quality and quantity of life, arguing against clinically important side effects, when pimobendan is used for treatment of canine heart failure. Furthermore, the necessity for even higher pimobendan dosages (≥0.3 mg/kg TID) in dogs that have entered ACVIM class D heart disease, have provided significant survival benefit and, in the authors' opinion, better quality of life (Ames et al., 2013). Even at very high (10 times recommended dosage) accidental over-dosing, adverse signs have been relatively modest in severity (Reinker et al., 2012). The database (November 2004 to April 2010) of an animal poison control center was searched for cases involving pimobendan toxicosis. Seven dogs that ingested between 2.6 mg/kg and 21.3 mg/kg were evaluated. Clinical signs consisted of cardiovascular abnormalities, including severe tachycardia (4/7), hypotension (2/7), and hypertension (2/7). In two dogs, no clinical signs were seen. All dogs were released from the hospital within 24 hours, although one died 3 days later.

As for all positive inotropic agents, pimobendan is *relatively* contraindicated in patients with outflow tract obstruction (e.g., HCM, subaortic stenosis, pulmonic stenosis). The inotropic effect may negatively affect dogs with early valvular lesions. In a study in asymptomatic dogs with mitral valve disease (Chetboul et al., 2007), pimobendan worsened mitral valve lesions and indices of cardiac function in dogs treated with pimobendan, compared to the group treated with the angiotensin-converting enzyme (ACE) inhibitor benazepril. The authors of the study proposed that the cardiotoxic effects were caused by an exaggerated pharmacodynamic effect, rather than from intrinsic toxicity. The importance of this finding has been diminished with the advent of the premature closing of the EPIC study (asymptomatic MMVD), discussed above.

Formulations and Dosing

Pimobendan is supplied as hard gelatin capsules (most countries) containing 1.25, 2.5, or 5 mg pimobendan. In the USA it is approved as a chewable formulation in four sizes: Vetmedin® 1.25, 2.5, 5, and 10 mg. It is not stable in suspension and should not be reformulated in this manner. The labeled dose recommendation is 0.25–0.3 mg/kg q 12 h. Initial efficacy may be enhanced by administration on an empty stomach but once steady-state is reached (a few days) it can be administered with food. Oral pimobendan solution (3.5 mg/ml and 1 mg/ml) is available in some countries (Bell et al., 2015; Yata et al., 2016). Levosimendan has been investigated as agent for treating heart failure in dogs. At this time, it is not available for clinical use.

Inotropic Agents: Inamrinone and Milrinone

Inamrinone (formerly amrinone) and milrinone are bipyridine derivatives commonly referred to as nonglycoside, noncatecholamine inotropic drugs. These compounds were discovered during an investigative search for cardiac stimulant agents that could be used to replace digitalis in the therapy of heart failure (Alousi et al., 1979). Numerous studies confirmed that inamrinone and milrinone evoke both a positive inotropic action in the heart and a peripheral vasodilator effect. The mechanism of action of the bipyridines is dissimilar to that of digitalis and does not involve adrenergic or other cell surface receptors. Rather, the cardiac inotropic and peripheral vasodilator actions of inamrinone and milrinone involve inhibition of the type III cyclic nucleotide phosphodiesterase enzyme. This enzyme is responsible for the selective metabolism of cAMP; hence, inhibition of type III phosphodiesterase by inamrinone or milrinone results in the accumulation of intracellular cAMP in cardiac and vascular tissues. Cyclic AMP subserves a positive inotropic response in myocardium and a vasodilator response in blood vessels. Because of concurrent inotropic and vasodilator actions, considerable attention has been focused first on inamrinone and later on milrinone as alternatives to digitalis in managing congestive heart failure patients (Mancini et al., 1985; Colucci et al., 1986a, 1986b).

Inamrinone (amrinone)

Although inamrinone increased cardiac contractile force and left ventricular pressure with relatively small changes in heart rate and systemic blood pressure in experimental dogs it has not been used clinically except as a last resort when other treatments have failed (Alousi et al., 1979). Adverse effects in people have prevented its routine use (Massie et al., 1985).

Milrinone

Milrinone is a structural congener of inamrinone, and the former is 20–30 times more potent than the latter. Initial studies suggested that milrinone might be relatively free of adverse side effects and could potentially be helpful in the management of heart failure in humans (Colucci et al., 1986a,b). However, studies in human patients with moderately severe heart failure indicated that milrinone was less effective than digoxin, and the combination of milrinone and digoxin was no more effective than digoxin alone. Moreover, milrinone administration was associated with an increased incidence of both ventricular and supraventricular tachyarrhythmias (DiBianco et al., 1989). In the OPTIME-CHF trial, milrinone administration had a bidirectional effect, worsening the outcome in patients with ischemic heart disease and having a neutral to beneficial effect in those with nonischemic heart disease (Felker et al., 2003).

Tachyarrhythmias were predictable as side effects of milrinone and other type III phosphodiesterase inhibitors inasmuch as their mechanism of action depends upon accumulation of cAMP. Hence, the initial enthusiasm for cardiac uses of milrinone and other type III phosphodiesterase inhibitors has decreased (Massie et al., 1985; DiBianco et al., 1989).

There is evidence that milrinone may be safe and effective in dogs with spontaneous heart failure (Kittleson et al., 1985b). Dogs may respond favorably to treatment with milrinone as the sole therapeutic agent. Positive effects were sustained for the 4 weeks of the study; however, heart failure worsened when milrinone was withdrawn but improved when the drug was reinstituted. The only apparent adverse reactions were asymptomatic ventricular dysrhythmias in two dogs. These investigators concluded that milrinone may be an effective drug for treating myocardial failure in the dog when administered orally twice daily in 0.5–1 mg/kg doses.

Clinical Use

Despite experience with oral milrinone in dogs, there is currently no approved oral formulation available. Milrinone lactate (Primacor®) is available as an injectable solution in a strength of 200 µg/ml. Milrinone is little used by veterinary cardiologists (Bonagura, 2010). Inamrinone is supplied only as an intravenous solution.

Angiotensin-Converting Enzyme Inhibitors: Enalapril Maleate and Benazepril, and Mineralocorticoid Receptor Blockers, Spironolactone

Recognition of the contribution of the renin–angiotensin–aldosterone system (RAAS) to the pathophysiology of congestive heart failure led to development of the angiotensin-converting enzyme inhibitors (ACEIs), such as captopril, enalapril, benazepril, ramipril, lisinopril, imidapril, temocapril, quinapril, alacecapril, etc. (Jackson, 2006).

Mechanisms of Action

Reduced renal perfusion during heart failure and other pathological and physiological situations evokes renin release from the juxtaglomerular apparatus (Figure 21.7).

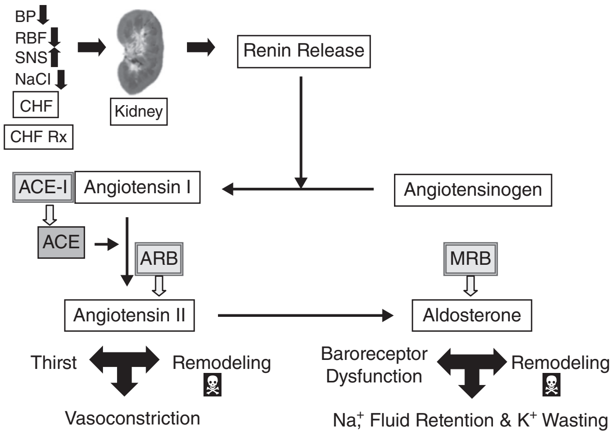

Figure 21.7 Reduction in blood pressure (BP), renal blood flow (RBF), and serum sodium concentration (NaCl), as well as activation of the sympathetic nervous system (SNS), the state of heart failure (CHF) and its treatment cause renin to be released by the juxtaglomerular apparatus, starting the cascade with angiotensinogen being converted to angiotensin I. This is followed by its conversion to angiotensin II by angiotensin converting-enzyme (ACE), and finally by stimulating the adrenal gland to secrete aldosterone. When chronic and excessive, the terminal hormones produced are toxic, particularly to heart failure patients, causing vasoconstriction, pathological remodeling of the myocardium and vessels, sodium and fluid retention, potassium wasting, and baroreceptor dysfunction. Blunting this cascade has become a major part of the treatment of cardiac disease in man and animals, using ACE-inhibitors (ACE-I), angiotensin II receptor blockers (ARB), and mineralocorticoid receptor blockers (MRB, e.g., spironolactone).

As discussed in Chapter 24, renin synthesizes the formation of angiotensin I from angiotensinogen. Angiotensin I is relatively inactive; however, it is cleaved by ACE into the potent vasoconstrictor and stimulator of aldosterone release, angiotensin II. Thus, by inhibiting ACE, ACEIs decrease the formation of angiotensin II and, through this mechanism, evoke peripheral vasodilation (relatively weak "mixed" or "balanced," arteriolar and venous, dilation). Angiotensin II-mediated release of aldosterone also is decreased by ACEI, thus facilitating sodium excretion and diuresis. Blockade of angiotensin II and aldosterone production blunts the negative effect of pathological remodeling of the heart, vasculature, and kidney in states of pathological RAAS activation (heart failure, renal failure, hypertension, and in response to off-loading therapies, such as vasodilators and diuretics)(Weber and Brilla, 1991; Schiffrin, 2006; Brown and Vaughan, 1998).

ACE is a relatively nonselective enzyme, not only converting angiotensin I to angiotensin II, but cleaving and thereby inactivating the bradykinin molecule as well. While the other effects of ACE are harmful in disease states, the increase in bradykinin is helpful as it blocks RAAS-induced vasoconstriction (via prostaglandins) and pathological remodeling.

The ACEIs commonly used in veterinary medicine, with the exception of captopril and lisinopril, are administered orally as prodrugs and converted to their active form in the liver (enalaprilat and benazeprilat). At this time, there is no clear superior ACEI, although captopril has clearly been surpassed in terms of convenience and because of its propensity to produce gastrointestinal upset.

Adverse Effects

ACEIs have the potential to produce symptomatic hypotension. This is due to the mixed vasodilatory effect of this group of drugs and is typically observed when ACEIs are used in conjunction with other off-loading therapies, such as vasodilators, diuretics, and sodium restriction. Hypotension is reversed by altering drug therapies but may be problematic in producing azotemia, inappetance, weakness, lassitude, and precipitating digitalis intoxication by reducing renal elimination. Other than at dosages 50–100 times those clinically recommended, the ACEIs are not directly nephrotoxic. The major impact of ACEI on the kidney, with clinically relevant dosages, is through production of hypotension, thereby reducing the kidney perfusion pressure and glomerular filtration rate, resulting in worsening of azotemia (MacDonald et al., 1987).

Less-common side effects include coughing and angioedema. While well-documented in people, there are no reports of these phenomena in animals. The cause for coughing is unclear, but is thought to be related to increased levels of bradykinin or resultant prostaglandins.

Veterinary clinicians have had experience with enalapril, captopril, benazepril, lisinopril, imidapril, alacepril, and ramapril. Of these, only enalapril has been extensively studied and is licensed for use in management of heart failure in the USA and benazepril has been marketed in Europe, Canada, South America, and Asia. We have learned through clinical experience with ACEIs (mainly captopril, enalapril, and benazepril in the USA) that their impact on kidney function is minimal, even in the face of severe heart failure. When azotemia is observed, ACEIs are usually administered in conjunction with diuretics and sodium restriction, often with resultant hypotension. Typically, diuretic cessation or reduction in the dosage results in the reversal of azotemia (Wynckel et al., 1998). In studies of enalapril in NYHA phase III and IV heart disease (moderate to severe heart failure), due to mitral valve regurgitation and DCM, there was actually a lower incidence of azotemia in the enalapril-treated group than the placebo-treated group (IMPROVE – Sisson et al., 1995; Cove Study Group, 1995; LIVE – Ettinger et al., 1998; Merck-Agvet, 1994). Furthermore, in a study of enalapril's role in the delay or prevention of heart failure due to naturally occurring MMVD, enalapril, at the standard dosage of 0.5 mg/kg daily, had no effect on serum creatinine concentrations as compared to placebo (Atkins et al., 2002). In fact, it is now well accepted that ACEI, administered chronically to both human and veterinary patients with naturally occurring and experimental renal failure, are beneficial (Abraham et al., 1988; Brown et al., 1999; Praga et al., 1992; Maschio et al., 1996; Grauer et al., 2000; Watanabe et al., 1999; Miller et al., 1999). Mechanisms for this improvement are postulated to be the antihypertensive effect, reduction of angiotensin II-induced mesangial cell proliferation, and renal vasodilatory effects of ACEI, the latter related to a fall in renal filtration pressure and proteinuria (Abraham et al., 1988; Praga et al., 1992; Maschio et al., 1996). Enalapril has recently been shown to reduce urine protein loss and reduce blood pressure in naturally occurring canine glomerulonephritis (Grauer et al., 2000). Likewise, benazepril reduced azotemia and proteinuria in a short-term study of experimental and naturally occurring renal insufficiency in cats(Watanabe et al., 1999) and lowered blood urea nitrogen (BUN) and creatinine concentrations and blood pressure in cats with polycystic kidney disease (Miller et al., 1999).

Efficacy

ACEIs represent a cornerstone in the chronic management of heart failure. By inhibiting the conversion of angiotensin I to angiotensin II (Jackson, 2006), they

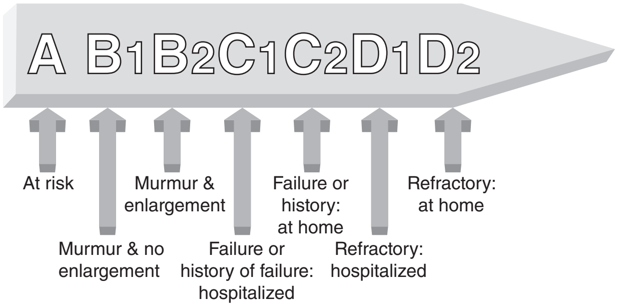

Figure 21.8 The American College of Veterinary Internal Medicine Cardiac Disease Classification scheme. Source: Atkins et al. 2009.

reduce the undesirable effects of angiotensin II (vasoconstriction; pathological remodeling with cardiomyocyte death and replacement; de novo fibrosis; and increased thirst) and aldosterone (pathological remodeling; sodium retention and potassium wasting; and baroreceptor dysfunction) recognized in congestive heart failure, hypertension, and glomerular disease.

They are indicated in virtually all cases of systolic heart failure. In subclinical MMVD (NYHA 1, ISAHC 1a and 1b, ACVIM B1 and B2) (Figures 21.8, 21.9, Table 21.4) RAAS suppression is known to be useful in asymptomatic dogs in ISAHC 1b, ACVIM B2, after there is cardiac remodeling (enlargement). ACEIs have proven useful in the management of systemic hypertension, clinical and subclinical cardiac disease, and proteinuric renal disease (Grauer et al., 2000; Atkins and Haggstrom, 2012; O'Grady et al., 2009).

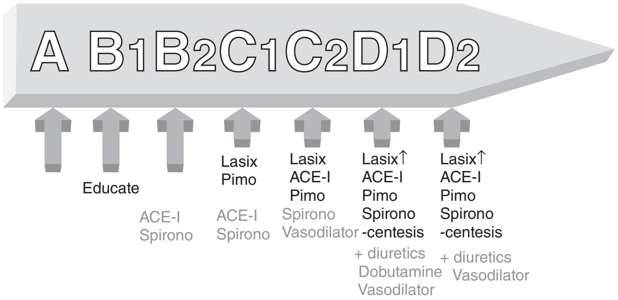

Figure 21.9 The American College of Veterinary Internal Medicine Classification Scheme with recommendations of the Consensus Committee on management of myxomatous mitral valve disease in dogs. Unanimous recommendations of the Committee are in bold black type, while the grey type represents the majority recommendations of that group, as well as the recommendations of the authors. Since the ACVIM Consensus Panel made its recommendations, two studies have been published which will likely impact this scheme. A European study demonstrated that the combination of benazepril and spironolactone were efficacious in treating dogs with heart failure (Bernay et al., 2010). The EPIC Trial demonstrated that pimobendan was effective in dogs in Stage B2 heart disease, prolonging the time to heart failure or cardiac death (Boswood et al., 2016). ACE-I, angiotensin converting-enzyme inhibitor; Spirono, spironolactone; Pimo, pimobendan; -centesis, thoracocentesis and/or abdominocentesis; Lasix, furosemide. Source: Atkins et al. 2009.

In NYHA phase III and IV heart disease in dogs (moderate to severe heart failure; Table 21.4), due to MMVD or DCM, enalapril improved survival by >100% as well as reducing pulmonary edema and, improving quality of life scores (IMPROVE – Sisson et al., 1995; Cove Study Group, 1995; LIVE – Ettinger et al., 1998). Exercise capacity is also improved in dogs with experimental mitral insufficiency (Hamlin et al., 1996).Benazepril has likewise been shown to improve survival (BENCH Study Group, 1999). The multicenter trials cited examined the ability of ACE inhibition to augment digitalis and diuretic therapy, rather than therapeutic benefits from enalapril alone. Nevertheless, these studies yielded convincing evidence that inhibition of ACE with enalapril improves quality of life and delays mortality in dogs with heart failure. It is clear that enalapril (and, likely the ACEI class) is beneficial in the management of heart failure when added to conventional therapy. There has been virtually no study of ACEI and inodilators being used together.

Preclinical heart disease: Enalapril has been studied in two placebo-controlled, randomized, double-blind clinical trials assessing its ability to slow the progression to heart failure in dogs with MMVD. The two studies were similarly designed, utilizing asymptomatic MMVD patients, the onset of pulmonary edema as the primary endpoint, and the ACEI, enalapril, versus placebo in a double-blinded, prospective trial (SVEP – Kvart et al., 2002; VETPROOF – Atkins et al., 2007). The SVEP trial and the VETPROOF had differing results because of differing study design. Both studies are useful to our understanding of the role of ACEI prior to the onset of heart failure in MMVD. Results of the SVEP trial argue strongly that, at the dosage evaluated, there is little to no benefit with ACE inhibition in a population of dogs with (107) and without (122) radiographic evidence of remodeling. The 5-year VETPROOF demonstrates a modest benefit in delaying the onset of heart failure with an additional 3-year follow-up substudy demonstrating a treatment benefit in delaying all-cause mortality; the latter study should ideally be repeated, with all-cause mortality as the primary endpoint. In the all-cause mortality substudy of 96 study participants followed to their deaths, it was demonstrated that a longer-term benefit (9 months) with ACEI therapy ($P < 0.02$) (Atkins and Keene, 2009).

Feline studies: In a retrospective study of cats presenting with HCM, enalapril therapy improved echocardiographic parameters (Rush et al., 1998). However, a larger prospective, blinded study (MacDonald et al., 2006), in which ramipril, administered for 1 year to Maine Coon and Maine Coon cross-bred cats with HCM, did not lead to a significant difference in LV mass, diastolic function, or BNP or aldosterone concentrations when were compared to controls.

Drug Interactions

A complete list of pharmacokinetic drug interactions is provided in the manuscript by Shionoiri (1993). Interactions that affect renal function are covered by Loboz and Shenfield (2005). ACEIs are used with other cardiovascular drugs (including diuretics) safely. However, ACEIs will potentiate the effect of diuretics and these drugs should be used together cautiously. Often times, the dosage of diuretic can (and should) be reduced with concomitant ACEI therapy.

When ACEIs are administered concurrently with nonsteroidal antiinflammatory drugs (NSAIDs), the latter may diminish ACEIs beneficial effect by blocking formation of prostaglandin, as some of the antihypertensive effect of ACEI is caused by generation of prostaglandins (Guazzi et al., 1998; Davie et al., 2000). NSAIDs also have been suggested to increase the risk of kidney injury (Loboz and Shenfield, 2005). Only one study examining this combination has been published for dogs, in which it was concluded in that tepoxalin did not alter renal function in healthy Beagle dogs receiving an ACEI (Fusellier et al., 2005). This has not been determined for other NSAIDs.

Use of ACE Inhibitors in Chronic Kidney Disease

As reviewed by Lefebvre and Toutain (2004), ACEIs can be beneficial in chronic kidney disease (CKD) because they reduce intraglomerular pressure, slow progression of lesions, and decrease proteinuria. These authors have documented the changes in disposition of ACEI in animals with renal impairment (Lefebvre and Toutain, 2004; Lefebvre et al. 2006). In dogs and cats, enalaprilat (active metabolite of enalapril) is predominantly cleared by the kidneys. Benazeprilat is excreted in the bile as well as in the urine, while enalaprilat is excreted solely by the kidneys. In the situation of renal dysfunction, benazeprilat serum concentrations are essentially unchanged from those expected in normal dogs (Toutain et al., 2000). Enalaprilat serum concentrations, however, with no alternative route for elimination, are elevated in the situation of renal dysfunction. Therefore, under these circumstances, benazepril is more predictable in terms of serum concentrations.

In cats with reduction in glomerular filtration rate, there was no alteration in clearance of benazeprilat(Lefebvre et al., 1999; Toutain et al., 2000; Brown et al., 2001).

ACE Inhibitors Currently in Use

Enalapril (Enacard®, Vasotec®, generic) has been approved for the treatment of heart failure in dogs in most countries, including the USA (Enacard®), but is no longer marketed in this country and the human generic drug is used. The dosage of enalapril used in the treatment of heart failure is 0.25–0.5 mg/kg q 12–24 h PO, whereas for systemic hypertension and proteinuria there is a wider rage of 0.25–1.0 mg/kg q 12–24 h PO. The dosage in cats is 0.25–0.5 mg/kg q 12–24 h.

Benazepril (Fortekor®, Lotensin®) has been approved for use in dogs in Canada, Europe (Fortekor®), South America, and Asia, and has been investigated in the USA for use in cats and dogs, at a dosage of 0.25–0.5 mg/kg q 12–24 h PO. Imidapril has been approved for the treatment of heart failure in dogs in the UK (Prilium®) at 0.25 mg/kg q 24 h PO. Ramipril (Altace®, Vasotop®) has been approved for use in dogs in the UK (Vasotop®). The dosage is 0.125–0.25 mg/kg q 24 h PO (starting at the lower end of the dose range and titrating upwards). Lisinopril (Prinivil®, Zestril®) has also been investigated in animals, but is not in common use. The dosage used in dogs is 0.5 mg/kg q 12–24 h, PO. The dosage in cats is 0.25–0.5 mg/kg q 24 h.

A combination of benazepril and spironolactone (Cardalis®) has now been approved for use in the EU for dogs in heart failure. This should provide a useful and convenient addition to the arsenal for management of heart failure, hypertension, and possibly proteinuric renal disease. US studies are in progress at the time of this writing. Cardalis® is supplied as 2.5 : 10, 5 : 20, 10 : 80 (mg benazepril : mg spironolactone) chewable tablets for once-daily use.

Mineralocorticoid Receptor Blockers

At the time of this writing, there are two mineralocorticoid receptor blockers on the market, eplerenone and spironolactone. Eplerinone is a human drug used to avoid some of the endocrine side effects of spironolactone. It is not used in veterinary medicine and is expensive, therefore will not be included in this discussion.

Spironolactone is a synthetic 17-lactone drug that is a competitive aldosterone receptor (mineralocorticoid receptor blocker, MRB). The result of this antagonism of mineralocorticoid receptor in distal renal tubule cells is an increase in urinary Na^+ and H_2O excretion and a decrease in K^+ excretion. In the dog, spironolactone is relatively quickly absorbed through the gastrointestinal tract into the plasma and is then converted to several active metabolites (Sadée, 1972; Karim et al., 1976). The bioavailability in the dog is highest when given with food, reaching 80–90% (Guyonnet et al., 2010). Using an experimental model of hyperaldosteronism, mimicking that in heart failure, Guyonnet and colleagues (2010) found that the spironolactone dosage of 2 mg/kg once daily restored the urinary Na^+/K^+ ratio to near normal.From this result and analysis of pharmacokinetic and pharmacodynamic data from this preclinical trial, the authors suggested that the optimal dosage of spironolactone in the dog is 2 mg/kg once daily.

As a weak, potassium-sparing diuretic (diuretics are discussed in Chapter 24), spironolactone was initially used to provide sequential nephron blockade and combat hypokalemia resulting from the use of loop and thiazide diuretics. However, current use focuses on the antagonism of aldosterone, which may have long-term benefits. Aldosterone has been implicated in the pathological remodeling (inflammation, hypertrophy, fibrosis) of cardiovascular and renal tissues (Ovaert et al., 2009; Hezzell et al., 2012). Because of this, the primary rationale for adding spironolactone as adjunctive therapy for heart failure is now mineralocorticoid receptor (specifically, aldosterone) blockade. Mineralocorticoid receptor blockade is now considered standard of care in humans with heart failure and low ejection fraction (McMurray et al., 2012; Yancy et al., 2013). The benefit seen with additional RAAS blockade in heart failure supports the concept of aldosterone breakthrough, discussed in Section Clinical Use. A similar study in 212 dogs with naturally occurring MMVD and heart failure showed a 69% reduction in risk of cardiac morbidity and mortality when spironolactone was added to standard therapy (ACEI, furosemide, with or without digoxin), as compared standard therapy alone (Bernay et al., 2010). A separate safety analysis of dogs involved in the aforementioned study showed that dogs receiving spironolactone, in addition to standard therapy, were not at higher risk of adverse events (death from renal disease, variations in serum Na^+, K^+, urea nitrogen, and creatinine) when compared to dogs receiving placebo and standard therapy (Lefebvre et al., 2013). Finally, spironolactone in combination with ACEI therapy appears to be safe when used to treat dogs with naturally occurring asymptomatic MMVD, as well those with occult DCM, without preexisting azotemia (Thomason et al., 2007, 2014).

Clinical Use

A consensus statement of the American College of Veterinary Internal Medicine, generating guidelines for the therapy of dogs with MMVD (Atkins et al., 2009), recommended the use of spironolactone as an adjunctive therapy in dogs with ACVIM stage D2 (refractory, with home therapy possible) heart failure (Figures 21.9 and 21.10). A majority of panelists also used spironolactone in the therapy of stage C2 (dogs previously stabilized with in-hospital heart failure therapy or clinical signs of heart failure, mild enough to be treated at home (Figure 21.9). A minority of panelists advocated the use spironolactone in dogs with ACVIM stage B2 (cardiac enlargement, but prior to onset of heart failure) MMVD.

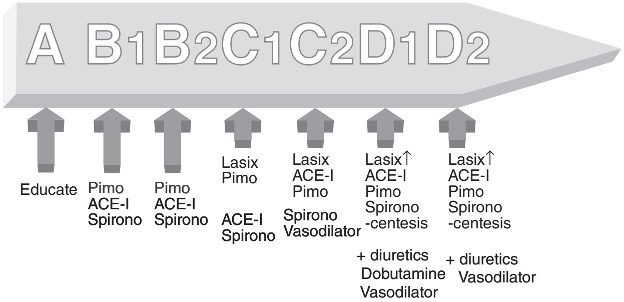

Figure 21.10 The American College of Veterinary Internal Medicine Cardiac Disease Classification scheme with the authors' treatment recommendations for dilated cardiomyopathy. See Figure 21.9 for abbreviations.

Spironolactone is approved within the European Union as adjunctive therapy in the treatment of canine heart failure, secondary to MMVD. Clinical trials are currently underway to evaluate the effects of spironolactone in combination with ACEI in the treatment of stage B2 and C1 MMVD dogs (ongoing US placebo-controlled, double-blind study of spironolactone and benazepril versus benazepril alone in dogs with MMVD and first-time heart failure, sponsored by CEVA Santa Animale with expected completion in 2017) (Borgarelli, 2011). There is no consensus regarding the use of spironolactone in other diseases, such as DCM and congenital heart disease, despite its having been used for decades to treat canine heart failure, secondary to a variety of causes.

Preliminary data from the authors' clinics suggest that aldosterone breakthrough occurs in dogs with heart failure due to naturally occurring heart disease and that the incidence is approximately 40%. Furthermore, it appears that aldosterone breakthrough may occur quite early in the disease course (Bomback and Klemmer, 2007; Lantis et al., 2014). Monitoring of urine aldosterone : creatinine (Gardner et al., 2007), may therefore help optimize an individual patient's pharmacotherapy and improve RAAS blockade (i.e., determine if spironolactone should be added as an adjunctive therapy).

Use in cats: Aldosterone has been found to be elevated in Maine Coon cats with asymptomatic HCM (MacDonald et al., 2006) and the use of spironolactone (2 mg/kg/day for 4 months) in the therapy of asymptomatic HCM in cats has been evaluated (MacDonald et al., 2008). Unfortunately, this study did not show reduction in left ventricular mass or improvement in an echocardiographic parameter of diastolic function and four of the 13 spironolactone-treated cats developed ulcerative facial dermatitis. A double-blinded, placebo-controlled clinical trial, evaluating the use of spironolactone in cats with heart failure due to HCM is currently under way (James et al., 2013). It is worth noting that, at interim analysis (n = 16 cats enrolled), no cat receiving spironolactone (1.3–2.2 mg/kg once daily) had experienced ulcerative facial dermatitis.

In conclusion, based on clinical and research data, the authors utilize spironolactone in virtually all instances in which ACEIs are employed. This would include dogs with ACVIM stage B2 MMVD (cardiac enlargement, but prior to onset of congestive heart failure (pulmonary edema); Figure 21.10); dogs with ACVIM stage C2 and D2 MMVD (at-home therapy of congestive heart failure; Figure 21.10); in systemic hypertension; in occult and florid DCM; and in proteinuric renal disease. At the current time, as data on MRB efficacy are pending, we do not feel that there is adequate evidence for the routine use of spironolactone in cats at this time.

Other Vasodilators: Prazosin, Hydralazine, Calcium Channel Blockers, Nitrovasodilators, Sildenafil, and Carvedilol

Vasodilators are characterized as: (i) "venous dilators" or "venodilators" (preload reducing), with nitroglycerin being a prime example – useful for signs of congestion; (ii) "arteriolar dilators" or "arteriodilators" (afterload reducing) include hydralazine and amlodipine – useful for low output signs (and for signs of congestion); and (iii) "mixed" or "balanced" vasodilators, which dilate both arterioles and veins, include the ACEIs, prazosin, pimobendan, and nitroprusside, useful for both congestive and low output signs. All, except pimobendan, also have the potential to cause hypotension and, hence, impaired renal perfusion. When used with other off-loading therapies (e.g., furosemide), hypotension and azotemia are more likely to result.

This group of drugs has been used extensively as treatment for heart failure to "off-load" or "unload" the failing heart (Hamlin, 1977; Zelis et al., 1979; Remme, 1993). The rationale is that decreasing the work load of the heart is better for the patient than administering a positive inotropic agent with toxic potential (i.e., digitalis) and which typically increases MVO_2 (inodilators, such as pimobendan, being an exception). If systemic arterial pressure (a component of left ventricular afterload) is reduced by a vasodilator drug, the left ventricle ejects blood into a circuit with lowered resistance. Further, peripheral venodilation diverts blood from the pulmonary to the systemic vasculature. This response is antagonistic to the formation of pulmonary edema, because it tends to restrict venous return to the heart (i.e., ventricular preload). Left ventricular size and wall tension decrease in response to reduction in ventricular preload and afterload. Myocardial oxygen demands decrease accordingly as the workload of the heart is reduced; cardiac output and hemodynamics should improve (Packer, 1984; Abrams, 1985).

Before initiating vasodilator therapy in treating congestive failure in animals, the clinician should be aware of potential problems; for example it has been assumed that drug-induced vasodilation would automatically increase peripheral perfusion and thereby increase oxygen availability to all tissues. However, vasodilator agents of the nitroglycerin type exert a predominant reduction in peripheral venous resistance as compared to arteriolar resistance. Pooling of blood in the venous capacitance beds in no way ensures increased perfusion of all tissues. Vasodilators are beneficial to the failing heart because they decrease cardiac workload, not by directly improving peripheral perfusion but because of vascular dilation. Furthermore, if arterial pressure is critically decreased, blood flow through the coronary and renal vascular beds may be compromised further. Reflex tachycardia accompanied by increased myocardial oxygen demand is another potential problem associated with fall in systemic blood pressure.

Prazosin

Prazosin (Minipress) is an α_1-adrenergic selective blocking agent (Chapter 7). Prazosin can be an effective vasodilator through the adrenergic blocking properties on vascular smooth muscle, but it is little used in veterinary cardiology. This drug may be useful in the treatment of renal hypertension (Zimmerman and Largent, 1983) and treatment of vesicourethral reflex dyssynergia in dogs (Haagsman et al., 2013). In dogs, prazosin undergoes hepatic metabolism with biliary excretion being the major route of elimination and 50% metabolized through first-past metabolism. Urinary excretion of this drug appears to be low (Rubin et al., 1979).

Hydralazine Hydrochloride

Hydralazine hydrochloride, USP (Apresoline®), is an arteriolar dilator that has undergone limited study in dogs with volume-overload heart failure (Kittleson et al., 1983; Häggström et al., 1996) and experimental heartworm disease with pulmonary hypertension (Atkins et al., 1994). Through its effect on systemic arterial beds, hydralazine reduces impedance, peripheral and pulmonary vascular resistance, and lowers impedance to left ventricular ejection. Stroke volume and cardiac output increase proportionately, thereby initiating hemodynamic improvement.

Beneficial effects of hydralazine are manifested in the management of congestive heart failure, secondary to MMVD. In this pathophysiological state, forward left ventricular stroke volume is reduced owing to a regurgitant fraction being pumped backward through the incompetent AV valve into the left atrium. By lowering systemic impedance to left ventricular ejection, hydralazine increases forward stroke volume and thereby reduces the regurgitant fraction. End-systolic volume and cardiac size are reduced because more blood is pumped from the cardiac chambers per beat. Reduction in cardiac size leads to commensurate decreases in wall tension (afterload) and myocardial oxygen consumption. Also, importantly, it reduces the size of the orifice of the incompetent mitral valve and hemodynamic improvement has been demonstrated in dogs with volume-overload congestive heart failure, caused by mitral valve regurgitation (Kittleson et al., 1983; Häggström et al., 1996). However, there are no outcome trials published to date. Hydralazine is likely effective in other volume overload states such as patent ductus arteriosus, aortic insufficiency, or septal defects.

Pharmacokinetics

Hydralazine is absorbed rapidly after oral administration in dogs, its onset of action is within 1 hour, and peak response at 3–5 hours. The drug undergoes extensive hepatic metabolism during its initial passage through the liver. Administration of hydralazine with food in dogs reduces its bioavailability (Semple et al., 1990). There is evidence that uremia in some way affects biotransformation of hydralazine, so that blood concentrations may increase in uremic patients.

Clinical Use

A recommended dosage for hydralazine in dogs involves the initial oral administration of 1 mg/kg; this dose can be adjusted upward, depending upon evidence of clinical improvement, but should not exceed 3 mg/kg. Although doses have been published for cats, it is not currently recommended for treatment.

The therapeutic response in dogs generally lasts 11–13 hours; thus twice-daily administration is suggested as the standard (Kittleson, 1983).

Adverse Effects

Important adverse effects of hydralazine therapy in humans are tachycardia and hypotension. It was reported that hypotension was not a problem in dogs when hydralazine dosage was titrated carefully against signs of clinical improvement; however, tachycardia does seem to be a common untoward development in dogs, treated with hydralazine (Kittleson et al., 1983). Because tachycardia increases myocardial oxygen consumption and may lead to cardiac decompensation, heart rate should be monitored during therapeutic implementation with hydralazine or any other vasodilating drug.

Concomitant administration of a beta-blocking drug might reduce the reflex tachycardia produced by hypotensive reactions to hydralazine. On the other hand, the potential negative inotropic response to cardiac beta-receptor blockade may exacerbate heart failure and/or worsen hypotension (see Chapter 7).

Calcium Channel Blocking Drugs

These agents suppress calcium ion (Ca^{++}) influx through plasma membrane channels in cardiac tissues, vascular smooth muscle, and other excitable cell types (Katz, 1985; Allert and Adams, 1987; Opie, 1984). The resulting decrease in intracellular Ca^{++} concentration leads to characteristic changes in physiological activity of affected tissues, including reduction in myocardial contractility, vasodilation in coronary and peripheral arterial beds, lowered impedance to left ventricular ejection, reduced myocardial oxygen demand, and slowed AV impulse conduction. Because of this diverse pharmacological profile, Ca^{++} channel blockers have been studied extensively for therapeutic application in a wide spectrum of cardiovascular disorders. Drugs of this group have been approved for the management of ischemic heart disease, hypertension, and some forms of cardiac dysrhythmias in human medicine. Other indications in people include obstructive cardiomyopathies, asthma, and cerebral ischemia (Stone and Antmann, 1983; Conti et al., 1985). Less is known about the clinical application of Ca^{++} channel blockade in veterinary medicine (Adams, 1986; Novotny and Adams, 1986; Johnson, 1985; Bright, 1992). The greatest experience has been with amlodipine, primarily in the management of systemic hypertension (Henik, 1997; Snyder, 1994) and with diltiazem, used to slow AV conduction (Gelzer et al., 2009) (thereby slowing ventricular response to atrial fibrillation or supraventricular tachycardia); to break supraventricular tachycardia; and as a lusitropic agent in cats with HCM (Bright, 1992). Amlodipine is also used in the treatment of heart failure to unload the ventricle(s), reducing cardiac size, reducing AV valvular insufficiency, improving forward cardiac output, and reducing impingement of an enlarged heart on the airways. The rationale for Ca^{++} channel blocking drugs in cardiovascular therapeutics in animals was summarized by Allert and Adams (1987). The use of Ca^{++} blocking agents as Class IV antiarrhythmics and electrophysiological properties in treating supraventricular tachyarrhythmias is addressed in Chapter 22.

Fundamentals of Ca^{++} Channel Blockade

Channel blockers are a heterogenous group of drugs that can be classified into two groups. The dihydropyridine calcium channel blockers (amlodipine, nifedipine) have greater vascular selectivity whereas the nondihydropyridine calcium channel blockers (diltiazem, verapamil) have greater selectivity for nodal and myocardial tissues. Their commonality is that they all selectively inhibit the L-type Ca^{++} channel – preventing its opening in smooth muscle and/or the myocardium. The dihydropyridine group (verapamil and diltiazem) are discussed more extensively in Chapter 22 of this book.

The calcium channels are protein moieties embedded within and spanning the permeability barrier of the phospholipid plasma membrane biolayer. Calcium channel blocking drugs induce negative inotropic effects in the heart by reducing transsarcolemmal influx of activator Ca^{++}.

Contraction of vascular smooth muscle is mediated by Ca^{++} and also depends on the influx of Ca^{++} through cell membrane channels (Somlyo, 1985). Therefore, Ca^{++} channel blockade in the vasculature evokes smooth muscle relaxation and a vasodilatory response. The resultant peripheral vasodilation, and accompanying decrease in peripheral vascular resistance, lowers impedance to left ventricular ejection, thereby reducing ventricular wall tension (afterload) during ejection.

Adverse Effects

Although the negative inotropic effects and vasodilator actions of Ca^{++} channel blockade can benefit hemodynamics by reducing cardiac workload, these same cardiovascular depressant responses obviously carry the risk of exacerbating hypotensive crisis. Adverse circulatory side effects of Ca^{++} channel blockade include a negative inotropic effect, with reduced cardiac output and hypotension, particularly with concurrent off-loading therapies (diuretics, ACEIs, vasodilators). This combination of effects can result in decompensation of preclinical or compensated heart failure Hypotension may also complicate treatment of patients *not* in danger of heart failure, resulting in lassitude, anorexia, collapse, and azotemia. Other potential side effects are sinus bradycardia and heart block, attributable to direct depression of sinoatrial firing rate and AV conduction, respectively.

Clinical Use

Antiarrhythmic applications are discussed in Chapter 22. For example, diltiazem is a common treatment for controlling heart rate in cats with HCM (Bright, 1992), and can be used in dogs for treating atrial fibrillation. When considered for HCM in cats, long-acting diltiazem is an attractive alternative. Based on the author's work (Johnson et al., 1996), conventional diltiazem should be administered at 7.5 mg TID and long-acting diltiazem at 45 mg q 24 h.

Dilacor® XR has the advantage of availability in capsules which contain three or four time-release tablets, as compared to the granules of the long-acting form. Based on studies with this product (Wall et al., 2005), either 30 or 60 mg of extended-release diltiazem tablets PO, once daily, can be administered, but Dilacor® XR administered at 30 mg BID can be effective without the untoward events.

Because other vasodilator drugs are available in veterinary medicine, (discussed in this chapter) amlodipine is rarely used in the management of heart failure. Amlodipine is the preferred drug for management of systemic hypertension in cats because it is longer-acting than other dihydropyridines (Snyder, 1994).

Nitrovasodilators: Nitroglycerin, Isosorbide Dinitrate, and Nitroprusside

The organic nitrates are nitrogen esters that exert their effect by acting as an exogenous source for nitric oxide. Nitric oxide is now known to be endothelium-derived relaxing factor (EDRF), an endogenous vasodilator. The nitrate vasodilators are esters of nitrous acid. They are metabolized to inorganic nitrite and denitrated metabolites.

Mechanism of Action

Nitrites, organic nitrates, and nitroso compounds all act to activate the enzyme *guanylate cyclase*. The resultant increase in intracellular cyclic-GMP (3,5-guanosine monophosphate) subsequently acts to inhibit contraction of vascular smooth muscle. This mechanism may involve a decrease in the availability of intracellular Ca^{++} in vascular smooth muscle cells or may interfere with the myosin–actin interaction (Opie and Gersch, 2009). Nitrates may also stimulate synthesis of the vasodilator prostaglandins, PGI_2 and PGE. Nitrates relax smooth muscle in both arteries and veins, but they are often used clinically as *preload* reducers. They decrease myocardial O_2 requirements (decrease workload of heart) and pool blood in the splanchnic vessels to reduce congestive signs in heart failure. They are commonly used to manage human patients with angina pectoris (chest pain caused by coronary artery disease), as coronary vasodilators and possibly by reducing cardiac work through reduction in preload.

Pharmacokinetics

Nitroglycerin and other nitrovasodilators are metabolized quickly with half-lives of only a few minutes. Nitrates have significant *first-pass effects*, and metabolites are one-tenth or less the potency of the parent drug. These drugs are, therefore, administered either topically, sublingually, or intravenously to avoid first-pass metabolism by the liver. In dogs receiving topical 2% nitroglycerin ointment applied to the pinnae at a dosage of 0.25 cm/kg or an equal amount of petrolatum, splenic dimension increased by an average of 7% in treated dogs as compared to no change in the placebo-treated dogs

(Parameswaran et al., 1999). Enlargement of the spleen was apparent at approximately 8 minutes, peaking at approximately 15 minutes. This occurred without elevation in splenic pressure, indicating relaxation of vascular smooth muscle in the spleen.

Nitrovasodilators in Clinical Use

Nitrovasodilators are available as ointments, creams, sublingual tablet, lingual spray, or buccal tablet.

Nitroglycerin (NitroBid®, Nitrol®, Minitran®, Nitro-Dur® and generic) is applied topically (as a 2% cream, 2% ointment, or 0.2, 0.4, 0.6, and 0.8 mg/h patch), The half-life is 1–3 minutes. An international group of veterinary cardiologists (ISACHC, 2006) recommends a topical nitroglycerin dosage of 4–15 mg q 6–12 h for dogs and 3–4 mg q 6–12 h for cats. It is usually applied to an area on the patient that lacks hair and where the patient will not lick it off (such as pinnae of ears or shaved portion of the body; most patients receiving nitroglycerin are not grooming). This topical formulation of nitrate vasodilation, administered TID, is preferred by the authors to supplement management of acute heart failure.

Isosorbide dinitrate (Isordil®) is available as an oral tablet (even though it has poor systemic availability), topical ointment, and lingual spray at a dosage is 2.5–5.0 mg/dog). Isosorbide mononitrate (IS-5-MN) may have better absorption and longer half-life. At both IV and PO doses of 3 mg/kg of IS-5-MN, the main metabolite of isosorbide-dinitrate, showed that the decrease in systolic blood pressure was closely correlated with the log plasma concentration (Sponer et al., 1984). Bioavailability was estimated at 71.5%. The half-lives for the distribution and elimination phases were approximately 6 minutes and 1.5 hours, respectively, the latter being only one-third of that obtained in man. Subsequently, dogs were given five different dosages of IS-5-MN orally (3.125–50 mg/dog) and showed a peak plasma concentration and area under the curve proportional to the dosage, whereas the terminal half-life did not differ markedly. The minimum plasma concentration for a hemodynamic effect was estimated to be 100 ng/ml.

Some studies in experimental dogs using oral administration of long-acting isosorbide dinitrate at 1–2 mg/kg had beneficial effects (Yamamoto et al., 2013). However, others have found less promising results. Adin and associates evaluated 5-isosorbide dinitrate in normal dogs and dogs in heart failure, using a dosage of 2, 3, and 4 mg/kg PO or placebo on separate days (Adin et al., 2001). Investigators were unable to detect a shift in blood volume with oral 5-isosorbide dinitrate administration at any dose tested in either group, despite adequate drug levels. The authors questioned the benefit of this compound in the treatment of dogs with congestive heart failure.

Sodium nitroprusside (Nipride®) is a potent, direct-acting mixed vasodilator, administered as a CRI because of its short half-life. Tolerance does not develop to nitroprusside, relative to other nitrate compounds, because it provides intracellular nitric oxide. It is administered, in 5% dextrose, at an infusion rate of 0.5–10 μg/kg/min (beginning at the lower dosage and titrating upwards, as needed) to veterinary patients, while monitoring blood pressure to avoid hypotension. The risk of hypotension can be lessened and additional benefit realized when used with dobutamine infusion or pimobendan PO or IV. It is supplemental to loop diuretic administration, lowering left and right heart filling pressures, systemic and pulmonary vascular resistance, ventricular afterload, and pulmonary edema. Cyanide toxicity can develop in patients in which renal elimination is compromised because sodium nitroprusside is rapidly metabolized to cyanide and thiocyanate.

Although there are limited publications on its effect and use in normal dogs or those in congestive heart failure, the ACVIM Consensus Committee on MMVD reached consensus on its use emergent congestive heart failure (ACVIM Stages C and D; Figure 21.8, 21.9) (Atkins et al., 2009). A small case series showed benefit with nitroprusside infusion in dogs with fulminant, unresponsive (furosemide, nitroglycerin, and oxygen, with no mention of ACEIs or inotropic agents) pulmonary edema due to MMVD (Greer et al., 2004). Eight of 10 dogs were released from the hospital, seven receiving from 1 to 3 (mean 1.75) μg/kg/h and one receiving 5 μg/kg/h. Infusion was reduced or stopped if blood pressure fell below 90 mmHg. Infusions were continued for an average of 14 hours and tapered over 2–3 hours.

Clinical Uses

In people, the organic nitrates are primarily used for the relief of anginal pain (angina pectoris) (Parker and Adams, 1977). Their use in veterinary medicine is limited because the dosage forms available can be inconvenient to use and the duration of action is brief. Nevertheless, they may be helpful for vasodilation and the acute treatment of pulmonary edema associated with congestive heart failure. Topical nitroglycerin ointment (2%) is typically used in a semiquantitative fashion:

cats: 0.3 cm strip (1/8 inch),
small dogs: 0.6–1.2 cm strip (1/4 to 1/2 inch),
medium dogs: 1.2–2.5 cm strip (1/2 to 1 inch),
large dogs: 2.5–5.0 cm strip (1 to 2 inch).

It is applied topically (usually to the inner ear flap) q 8 h, for the acute management of congestive heart failure. After 24 hours, because of concerns or tolerance, the drug is stopped if the patient's condition allows, or withdrawn for 8 hours and then continued at the same dosage at "8 hours on – 8 hours off". The drug is rarely dispensed but, if so, it can be used to treat orthopnea in heart failure patients, by administering it at bedtime once daily. Owners should be instructed to wear exam gloves when applying the ointment and to wash hands thoroughly afterwards.

Tolerance

Tolerance to nitrovasodilators develops with repeated administration. The mechanism is suspected to be a progressive depletion of sulfhydral groups necessary for the formation of nitric oxide. Efficacy is improved if the drug is used intermittently instead of continuously because intermittent use allows time for regeneration of sulfhydral groups. Optimum intermittent use is to provide a nitrate free interval of 8 hours or more during the day.

Adverse Effects

The most common and limiting side effect of these drugs is hypotension. Methemoglobinemia can occur with accumulation of nitrites but, both this and cyanide poisoning are extremely uncommon, as this therapy is usually used in the short-term management of acute heart failure requiring hospitalization.

Phosphodiesterase V Inhibitors: Sildenafil

Sildenafil (Viagra®) is an orally active PDE V inhibitor. Phosphodiesterase V is found in relatively high concentration in lung and penile erectile tissue and levels are elevated in humans with pulmonary hypertension (PHT). Type V phosphodiesterase inhibitors prevent degradation of cyclic guanosinemonophosphate-specific phosphodiesterase-5 (cGMP), resulting in relaxation of smooth muscle in pulmonary vasculature and, to a lesser degree, systemic vessels. This enhances nitric oxide-mediated pulmonary vasodilation and may provide additional beneficial effects on vascular remodeling and cardiac function (Takimoto et al., 2005). However, a canine study looking specifically at sildenafil's effect on cardiac function did not demonstrate inotropic benefit (Dias-Junior et al., 2006).

Pulmonary hypertension, a clinically important disease associated with high morbidity and mortality in dogs, is most often a sequelae of other disease processes (chronic obstructive pulmonary disease and heartworm disease), thereby requiring a balanced therapeutic approach which targets both the underlying disease and palliation of clinical signs. An important goal of therapy is to reduce pulmonary artery pressures. Early vasodilators, however, have had no preferential effect on pulmonary vasculature and thus have had questionable benefit, as well as the potential to worsen the clinical signs of pulmonary hypertension by producing systemic hypotension.

Sildenafil is currently the most extensively researched of the PDE V inhibitors, and has been shown to improve erections, as well as exercise tolerance and quality of life in humans with PHT, resulting in FDA approval for its use in erectile dysfunction and PHT. Although, still expensive, sildenafil is now available as a generic preparation.

Pharmacokinetics

There are little published data on sildenafil in the dog. Incompletely described pharmacokinetic properties include T_{max} of 1 hour or less. Bioavailability is attenuated by presystemic hepatic metabolism. The volume of distribution is 5.2 l/kg, with plasma protein binding of 84%. The dog has an elimination half-life of 6.1 hours. After single oral or intravenous doses of [^{14}C]-sildenafil, the majority of radioactivity was excreted in the feces (Walker, 1999; Sugiyama, 2001; Al-Mohizea et al., 2015).

In normal human subjects, sildenafil is rapidly absorbed after oral administration, with absolute bioavailability of about 40%, due to the extensive first-pass metabolism. It is eliminated predominantly by hepatic metabolism (mainly by cytochrome P450 3A4) and is converted to an active form. Sildenafil and its metabolite have terminal half-lives of approximately 4–5 hours. The maximum observed plasma concentration of sildenafil is reached within 30–120 minutes (median 60 minutes) of an oral dosing in a fasting state (Al-Ghazawi et al., 2010).

Efficacy

Well-controlled dose studies of vasodilator effects of sildenafil are lacking in the veterinary literature; however, there are three small clinical studies, one being placebo controlled (Dias-Junior et al., 2006; Bach et al., 2006; Kellum and Stepien, 2007). The dose used is approximately 2 mg/kg sildenafil every 8–24 hours. These studies show improvement in pulmonary hypertension or quality of life scores.

In naturally occurring PHT in dogs with concurrent MMVD, dosage of 1 mg/kg TID sildenafil (Brown et al., 2010), with concomitant but variable use of furosemide, enalapril, pimobendan, digoxin, spironolactone, and thiazide diuretics, produced improvements in pulmonary pressures. Exercise capacity was significantly greater and quality of life scores significantly higher in dogs receiving sildenafil than in dogs receiving placebo. The authors concluded that sildenafil decreases systolic pulmonary arterial pressure in dogs with PAH and its use is associated with increased exercise capacity and quality of life, when compared to treatment with placebo. No adverse effects were noted.

Because of the variability and complexity of the potential causes of PHT, additional harmful effects of the underlying disease, and the systemic response to pulmonary hypertension (e.g., congestive heart failure), sildenafil is often used in combination with other medications, including conventional heart failure therapeutics, such as diuretics, ACEIs, and pimobendan (Hoskins, 2006). While adverse side effects with long-term therapy have been minimal or unrecognized, long-term studies are lacking.

Carvedilol

This compound has been approved for use in humans to treat hypertension and heart failure. Antiarrhythmic effects are discussed in Chapter 22. It is both a nonselective β_1 and β_2-receptor antagonist and an α_1-receptor antagonist (see Chapters 7 and 22 for more complete discussion). The ratio of β_1/β_2 to α_1-receptor antagonist potency for carvedilol is 10 : 1. Thus, this agent should reduce myocardial workload by lowering heart rate and peripheral vascular resistance. Carvedilol is also an antioxidant and its beta-blocking effects have potential beyond heart rate control and vasodilation. It is well accepted in human medicine that beta-blockers (carvedilol and others) play a role in heart failure management, improving survival, quality of life, cardiac health, and exercise capacity (Packer et al., 1996).The mechanism for this is the blunting of SNS activity, because SNS has multiple harmful effects, including: production of tachycardia and arrhythmias; vasoconstriction with increased afterload; RAAS activation; and myocardial remodeling, apoptotic cell death, and fibrosis.

Uechi et al. (2002) reported that in dogs with experimental mitral valve regurgitation, carvedilol (0.2 mg/kg) decreased heart rate, whereas renal function, arterial blood pressure, and left ventricular contractile function were unaffected. Carvedilol (0.4 mg/kg) decreased heart rate, blood pressure, and renal function. The tachycardic response to isoproterenol was significantly diminished for 36 hours by 0.4 mg/kg carvedilol versus 24 hours with 0.2 mg/kg. The authors determined that carvedilol should be initiated at less than 0.2 mg/kg and titrated up to 0.4 mg/kg for dogs in heart failure.

Because of beta-blockers' negative inotropic effect, they are typically (or always) started prior to heart failure or at least during remission. The dosage is gradually increased until the target (25 mg BID in DCM, or approximately 0.5–1 mg/kg) is reached or the dog can no longer tolerate the drug. If the latter occurs, the dosage is backed off to the previously tolerated dosage and maintained or again carefully increased once stabilization has occurred. Carvedilol and other beta-blockers are typically used in conjunction with other agents used in treating heart failure. Preliminary studies in dogs with MMVD show promise with this agent (Arsenault et al., 2005; Gordon et al., 2005, 2006); however, absorption of carvedilol varies markedly between individuals, making dosage determination difficult (Uechi et al., 2002). Furthermore, the only placebo-controlled, double-blind study of a beta-blocker (bisoprolol) in veterinary medicine failed to show a benefit in slowing the progression to heart failure in dogs with asymptomatic MMVD (Keene et al., 2012). The use of beta-blockers in DCM is still advocated, though their use has not been evaluated in dogs with this affliction.

Ancillary Therapy in Congestive Heart Failure

Diuretics

Diuretics are covered in Chapter 24. The use of potent loop-acting diuretics (furosemide, torsemide, bumetanide) are the mainstay of the management of congestive heart failure in man and animals. Today, in dogs, their use is supplemented with ACEIs and pimobendan in most cases ("triple therapy"). The authors advocate "quadruple therapy," adding spironolactone to any drug regimen, which employs ACEIs. This is because of the phenomenon termed aldosterone breakthrough, described in Section Mineralocorticoid Receptor Blockers, Clinical Use. Furthermore, the authors do not prescribe furosemide as a monotherapy, because of its RAAS-activating properties, similar to that of vasodilators. An ACEI virtually always accompanies the use of furosemide in the authors' clinics.

Patients receiving diuretics should be carefully monitored. Pronounced diuresis can reduce blood volume to the extent that dehydration lowers preload, such that ventricular performance is inadequate. A reduced ventricular filling pressure (preload) is good on the one hand because it reduces wall tension, myocardial oxygen demand, and the propensity for edema formation. Conversely, this reduction in venous return – without concurrent positive inotropic effects – may well lead to reduced cardiac output, which results in reduction in renal perfusion, azotemia, and reduced clearance of certain drugs. Furthermore, inappetance and/or vomiting contribute further to dehydration and electrolyte loss. Hypokalemia is one of the more important concerns in this setting, as potassium levels fall with anorexia, polyuria, excessive diuresis, and emesis. This is, however, counteracted with potassium supplementation, ACEIs, and MRB (spironolactone). Serum electrolyte and renal values (BUN and serum creatinine) should be monitored, along with body weight, state of hydration (skin turgor, packed cell volume, total protein, serum albumin and sodium).

Overview of the Management of Cardiovascular Disease

Type of Heart Disease and Related Hemodynamic Abnormalities

The sympathetic nervous system and RAAS are both activated in heart failure and even before its onset. In virtually all cases, this activation is detrimental to cardiac patients.

In MMVD, contractility is at least partially maintained until the patient reaches terminal stages of illness. But recent studies of pulmonary transit time of blood (time required to traverse the lungs and reach the left atrium) indicate that subtle changes are present much earlier. These dogs have generally not been thought to require inotropic support but, today, pimobendan has been shown to be useful in the management of MMVD-induced congestive heart failure.

In heart failure, preload and afterload are both elevated and should be reduced. Heart rate varies but is usually high and can be reduced with digoxin, for example, and simply by managing heart failure and normalizing the autonomic forces on the cardiovascular system.

Dogs with DCM have similar abnormalities except that contractility is always diminished and often, profoundly so. This requires inotropic support in the emergency room with dobutamine CRI, digoxin IV or PO, or IV or PO pimobendan and chronically with pimobendan PO, with or without digoxin (Figure 21.10).

Cats with HCM suffer diastolic failure and increased preload and afterload, often with elevated heart rate. Afterload increase is not as severe as in eccentrically hypertrophied hearts, as discussed above for MMVD and DCM, because the concentric hypertrophy of HCM (and hypertension) is adaptive in terms of afterload (LaPlace relationship, in which chamber radius increases and wall thickness reduces afterload). Systolic function is thought to be adequate in most cases. Therapy is different with treatment of rapid heart rate, diastolic failure (lusitropic and negative chronotropic drugs), and congestion.

Pressure overload, due most often to systemic hypertension, pulmonary hypertension (heartworm disease, etc), and obstructive disease (SAS, PS, coarctation of the aorta, etc.), also produces heart failure with increased afterload, normal, elevated or decreased preload, and increased heart rate, as in heart failure of other causes.

Severity of Heart Disease

There are numerous classification schemes in veterinary medicine for the categorization of severity of cardiac disease (Atkins and Haagstrom, 2012). Two of these (Figure 21.8), the ISACHC (International Small Animal Cardiac Health Council) and ACVIM (American College of Veterinary Internal Medicine), are most commonly in use today (ISACHC, 1994; Atkins et al., 2009).

The ISACHC classification consists of four categories (Ia, Ib, II, and III), which are schematized in Figure 21.6 and described in Table 21.4. The ACVIM classification of cardiac disease, which was adapted from the American College of Cardiology/American Heart Association classification system, depicted in Figure 21.8, uses an A through D categorization of cardiac disease and does not rely heavily upon exercise tolerance as a criterion, a weakness of previous schemes. It also includes a category (A) for dogs without heart disease but who are at risk (e.g., Cavalier King Charles Spaniels at risk to develop MMVD). Category B includes dogs with mild heart disease, without (B1) and with (B2) cardiomegaly, but without present or historical evidence of congestive heart failure. Category C includes dogs with signs of heart failure, either hospitalized (C1) or treated at home (C2) and is similar to category D, which includes refractory or end-stage heart failure patients, treated in the hospital (D1) or at home (D2). Obviously, this categorization is fluid, with patients able to move back and forth between the stages of heart disease, with decompensation, with disease progression, and with successful treatment.

Choice of Pharmacological Agents, Based on Signs

One can also pick agents based on pathological responses seen in individual patients, for example, congestion, tachycardia, poor systolic function, and arrhythmia. In Tables 21.1 and 21.2 drugs are classified as to the type of abnormality for which they are likely to be indicated, with the suggested dosage.

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22

Antiarrhythmic Agents

Kathryn M. Meurs and Jim E. Riviere

The authors thank HR Adams for the original chapter upon which this is based.

An arrhythmia is typically defined as an abnormality in the rate, regularity, or site of origin of the electrical impulse of the heart, or a disruption in impulse conduction such that the normal sequence of atrial and ventricular activation is changed. In many cases cardiac arrhythmias have no clinical importance, and in some cases are considered normal findings (sinus arrhythmia in the dog). However, arrhythmias that lead to very slow or rapid heart rates or very irregular heart rates can have significant clinical implications, particularly if cardiac disease is present. The decision to treat a cardiac arrhythmia with an antiarrhythmic should be based on many factors including heart rate, type of arrhythmia, presence or absence of clinical signs such as syncope or exercise intolerance, and presence of underlying heart disease. Arrhythmias in the face of underlying heart disease, particularly with myocardial dysfunction (dilated cardiomyopathy, etc.) can be likely to lead to the development of clinical signs, including sudden death. Although there are many antiarrhythmics, only a small number have been well studied and shown to be effective in veterinary medicine and this chapter will concentrate on those. This chapter focuses on the more common antiarrhythmic agents used in veterinary medicine and introduces their principal pharmacodynamic actions on cardiac rate and rhythm.

Rhythmicity of the Heart

Normal cardiac rhythmicity is maintained by (i) dominance of a single pacemaker discharging regularly with the highest frequency, (ii) rapid and uniform conduction through normal routes of impulse conduction, and (iii) long and uniform duration of the action potential and refractory period of cardiac myofibers. In addition, duration of the Purkinje fiber action potential normally outlasts that of the ventricular muscle, thus providing a safety factor preventing reentry and reexcitation of the Purkinje system by the muscle action potential. A disturbance in any of the preceding factors can be arrhythmogenic, for example an inappropriate increase in automaticity of normally latent pacemaker cells, abbreviation of the refractory period, slowing of conduction velocity, or disparate refractory periods of adjacent fibers. Likewise, treatment of arrhythmias with the drugs discussed in this chapter is aimed at correcting these disturbances by altering cardiac pacemaker threshold, sodium, potassium, and/or calcium currents.

Arrhythmias often are associated with: imbalance of the parasympathetic and sympathetic branches of the autonomic nervous system; changes in serum electrolyte concentrations, especially potassium and calcium ions (K^+ and Ca^{++}); hypoxemia; acidosis; changes in concentration of carbon dioxide; excessive stretch of cardiac tissue; mechanical trauma; myocardial disease states such as congestive heart failure and viral myocarditis; numerous drugs; and ischemia and infarction of the heart muscle. Chapters 6, 7, and 8 on the autonomic system and the introduction to cardiac function in Chapter 21 should be consulted for an appropriate background in these areas.

Hemodynamic instability occurring during cardiac arrhythmias results from alterations in heart rate, changing the regularity of heartbeats, and losing atrial assistance in ventricular filling. Electromechanical synchrony of the cardiac chambers is thereby lost, culminating in ineffectual filling and ejection of the ventricles and hemodynamic deterioration of the patient. Antiarrhythmic drugs suppress arrhythmias and help restore hemodynamic stability by altering basic electrophysiological processes in the heart.

Electrophysiological Properties of Cardiac Cells

The classification system for clinically useful antiarrhythmic drugs is based mainly on the predominant pharmacological effects of a drug on the action potential of cardiac cells (Vaughn Williams, 1984; Adams, 1986). Accordingly, a useful understanding of antiarrhythmic drug actions and affiliated nomenclature depends first on

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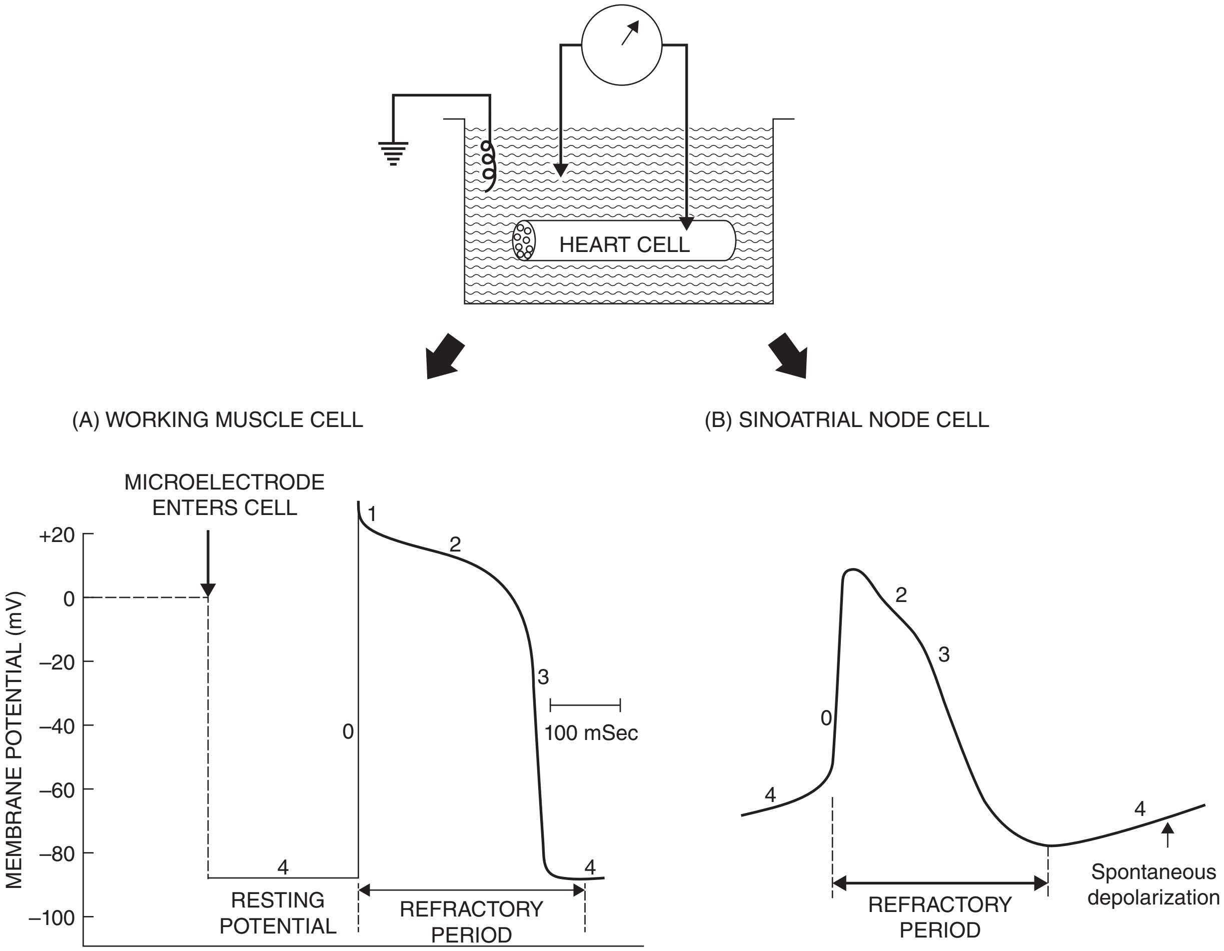

Figure 22.1 Cardiac action potentials recorded from a working myocardial cell (A) and a sinoatrial pacemaker cell (B). The nonautomatic working muscle cell (A) exhibits a constant phase 4 resting potential during diastole, whereas the automatic cell (B) undergoes spontaneous depolarization during phase 4, leading to threshold and spontaneous excitation. The cell is inexcitable or poorly responsive to additional stimuli during much of the action potential, and this refractory period helps prevent premature excitation. See text for further details. Source: Adams, 1986.

a good comprehension of basic bioelectric properties of the heart. An overview of salient features of this topic is outlined here relative to action potentials of cardiac cells and types of cardiac arrhythmogenesis.

Action Potentials of Cardiac Cells

The electrical activity of individual heart muscle cells can be recorded with a microelectrode capable of entering the intracellular space of a single cell, as shown schematically in Figure 22.1. Some of the common terms used to describe the configuration and ionic determinants of cardiac action potential components are defined here (Adams, 1986):

1) Membrane potential is the voltage difference across the cell membrane, that is the difference in electrical voltage between the intracellular and extracellular spaces. By convention, the resting membrane potential is defined as the charge inside the cell relative to the extracellular side, in which case the resting potential is a negative charge. An increase in resting membrane potential would therefore designate a more negative intracellular charge (e.g., an increase from −70 to −90 mV), while a decrease in resting membrane potential would designate a less negative intracellular charge (e.g., a decrease from −70 to −50 mV).
2) Depolarization is the loss or decrease in electronegativity of the intracellular space, for example a decrease in membrane potential from −90 to −50 mV (partial depolarization) or from −90 to 0 mV (complete depolarization).
3) Hyperpolarization is an increase in electronegativity of the intracellular space.
4) Inward current is the change in electrical charge across the cell membrane that results from influx of positively charged ions or, alternatively, from efflux of negatively charged ions.

5) Spontaneous depolarization of automatic cells is a physiological and progressive decrease in resting potential during diastole, leading spontaneously to threshold and automatic firing.
6) Threshold potential is the membrane potential required for excitation of the cell, initiating the action potential and affiliated cellular responses.
7) Phase 0 is the rapid depolarization phase of the action potential of the excited cell, mediated by a rapid inward current carried by Na^+ through fast sodium channels of the cell membrane.
8) Phase 1 is the initial early repolarization phase of the action potential.
9) Phase 2 is the plateau phase of the action potential, mediated in part by a slow inward current carried by Ca^{++} through slow calcium channels of the cell membrane.
10) Phase 3 is the rapid repolarization phase of the action potential, returning membrane potential to the diastolic level.
11) Phase 4 is the membrane potential during diastole; it is constant in working muscle cells but undergoes spontaneous depolarization in cells with automaticity.
12) Refractory period is that early and late interval of the action potential during which excitability of the cell is essentially absent (functional refractory period) or depressed (relative refractory period), respectively.
13) Depressed fast sodium ion (Na^+) responses are slowly rising phase 0 depolarizations due either to premature excitation during the relative refractory period of normal cells or excitation of sick cells with low diastolic potentials; depressed fast Na^+ response action potentials develop cardiac impulses that propagate poorly with reduced conduction velocity.
14) Slow Ca^{++} responses are analogous to the slow inward Ca^{++} current during phase 2; this term is used to describe the very slowly rising phase 0 depolarizations mediated by Ca^{++} when the fast Na^+ channels are inoperative. Slow Ca^{++} action potentials develop cardiac impulses that propagate poorly with extremely slow conduction.

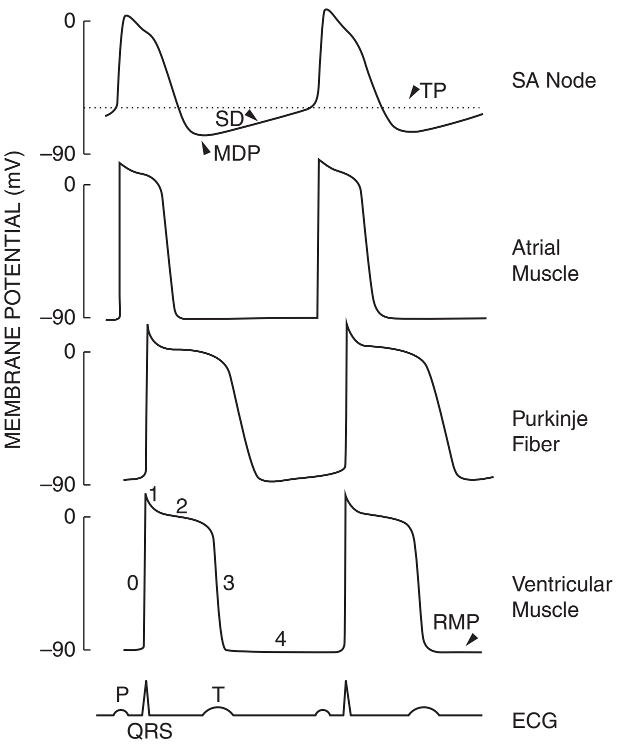

Figure 22.2 Schematic diagrams demonstrating the temporal relationships between transmembrane action potentials recorded from cells of the sinoatrial node (SA), atrial muscle, Purkinje fibers, and ventricular muscle (see text for discussion). Modeled after Trautwein, 1963. Source: Adams, 1986.

When a cardiac cell is stimulated, the electrical potential measured across the cell membrane undergoes a depolarization and repolarization cycle that can be differentiated into five sequential components. These components are referred to as phases 0, 1, 2, 3, and 4 (Figure 22.1). The precise morphology of the 5 phases of the cardiac action potential varies with the anatomic region of the heart. A schematic diagram illustrating the configuration of action potentials derived from sinoatrial (SA) tissue, atrial muscle (AM), Purkinje fibers (PF), and ventricular muscle (VM) is depicted in Figure 22.2 along with corresponding waveforms of the electrocardiogram (ECG). Action potentials of a sinoatrial pacemaker cell (Figure 22.1B) and a typical working heart muscle cell (Figure 22.1A) will be addressed as examples of cardiac tissue with and without normal automaticity, respectively.

Working heart muscle cells: Electrical diastole is designated by phase 4 of the cardiac action potential (Figure 22.1A); this resting membrane potential is steady at about −90 mV. Polarization across the cell membrane is maintained primarily because of the unequal distribution of K^+ inside and outside the cell. The Na^+,K^+-adenosine triphosphatase transport system maintains high intracellular K^+ relative to extracellular K^+, and the cell membrane is selectively permeable to K^+ during phase 4 diastole when compared to other ions such as Na^+ or Ca^{++}. When the cell is stimulated to its particular threshold level, however, the selective permeability characteristics of the cell membrane to K^+ are momentarily lost. Other ions now cross the sarcolemma and produce the typical depolarization–repolarization cycle that comprises the action potential (Figure 22.1).

Phase 0 of the action potential reflects the extremely rapid depolarization spike produced by Na^+ rushing into the cell through specific “fast Na^+ channels” or passageways of the sarcolemma. Phase 0 is terminated as early (phase 1) and delayed (phase 3) repolarization occur, restoring the membrane potential to its resting diastolic level of phase 4 (Figure 22.1). The cell is refractory to additional stimuli during the early and intermediate phase of the action potential cycle; it is only partially responsive if stimulated prior to complete repolarization and return to normal phase 4 diastolic potential.

The phase 2 plateau of the action potential partially represents a brief anomalous delay in restoration of K^+ permeability (Figure 22.1). A critically important component of phase 2 comprises an influx of Ca^{++} through specific “slow Ca^{++} channels” or “slow cation channels” of the cell membrane. This slow inward Ca^{++} current is the mechanism whereby membrane excitation is coupled to activation of the contractile elements of heart muscle cells (Parker and Adams, 1977). The influx of Ca^{++} during phase 2 triggers a release of greater amounts of Ca^{++} from intracellular storage sites, and the increased cytosolic Ca^{++} proportionately activates the contractile machinery of the myocardial cells.

Sinoatrial pacemaker cells: Unlike working myocardial cells, automatic cells do not exhibit a clearly definable resting membrane potential during phase 4. Instead, phase 4 is characterized by a slow spontaneous depolarization to threshold potential (Figure 22.1B), thereby discharging automatically and leading into the more rapid depolarization of phase 0. However, the slope of phase 0 depolarization of SA pacemaker cells is much less than that of working muscle cells (Figures 22.1, 22.2). This distinction may be explained by a component of slow Ca^{++} influx in the genesis of phase 0 depolarization in these types of automatic cells (Adams, 1986). Cells with normal automaticity (i.e., spontaneous phase 4 depolarization) also are found in specialized atrial conduction tracts, the distal region of the AV node, AV valves, and PF.

Classification of Arrhythmogenic Mechanisms

The basic mechanisms involved in genesis of cardiac arrhythmias involve abnormalities of impulse formation (i.e., arrhythmias caused by changes in automaticity), impulse conduction (i.e., arrhythmias caused by reentry phenomena), and a combination of automaticity and reentry (Singh et al., 1980; Binah and Rosen, 1984).

Disturbances in Automaticity

The action potential from the SA node, AM, PF, and VM are shown in Figure 22.2. The five phases of the action potential (0, 1, 2, 3, 4) are numbered in the first complex of VM. Notice spontaneous depolarization (SD), maximal diastolic potential (MDP), and threshold potential (TP) in the automatic cells of SA and PF. The resting membrane potential (RMP) is shown in the nonautomatic cells of the AM and VM. The P wave of the ECG corresponds to depolarization of SA and AM, while the QRS complex and T wave correspond to depolarization and repolarization, respectively, of ventricular cells (Figure 22.2).

Automatic cells of the SA node normally are the dominant pacemaker, reaching threshold first with the resultant propagating impulse exciting all other potential pacemaker cells before they spontaneously attain threshold values (Figure 22.2). If automaticity of the SA node is depressed or the spontaneous firing rate in some other tissue (latent pacemaker) is accelerated, regions of the heart other than the SA node may serve as the pacemaker and initiate ectopic impulses. Examples are shown in Figure 22.3.

Automaticity is enhanced when the slope of phase 4 SD is increased (e.g., from a to b in I of Figure 22.3); this decreases the time required to reach TP, thereby increasing the frequency of spontaneous discharge. The result is an increase in heart rate when the SA pacemaker is involved or emergence of ectopic beats if a normally latent pacemaker is involved. By decreasing the slope of spontaneous depolarization (e.g., from b to a or from a to

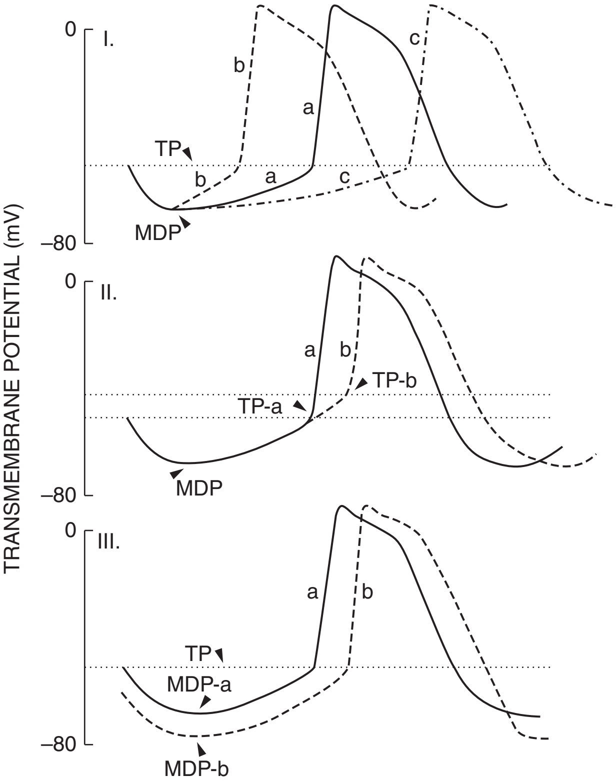

Figure 22.3 Schematic representations of transmembrane action potentials of cardiac cells with the property of automaticity and potential mechanisms whereby antiarrhythmic drugs can influence automaticity (see text for discussion). Source: Adapted from Hoffman and Cranefield, 1960; Mason et al., 1973.

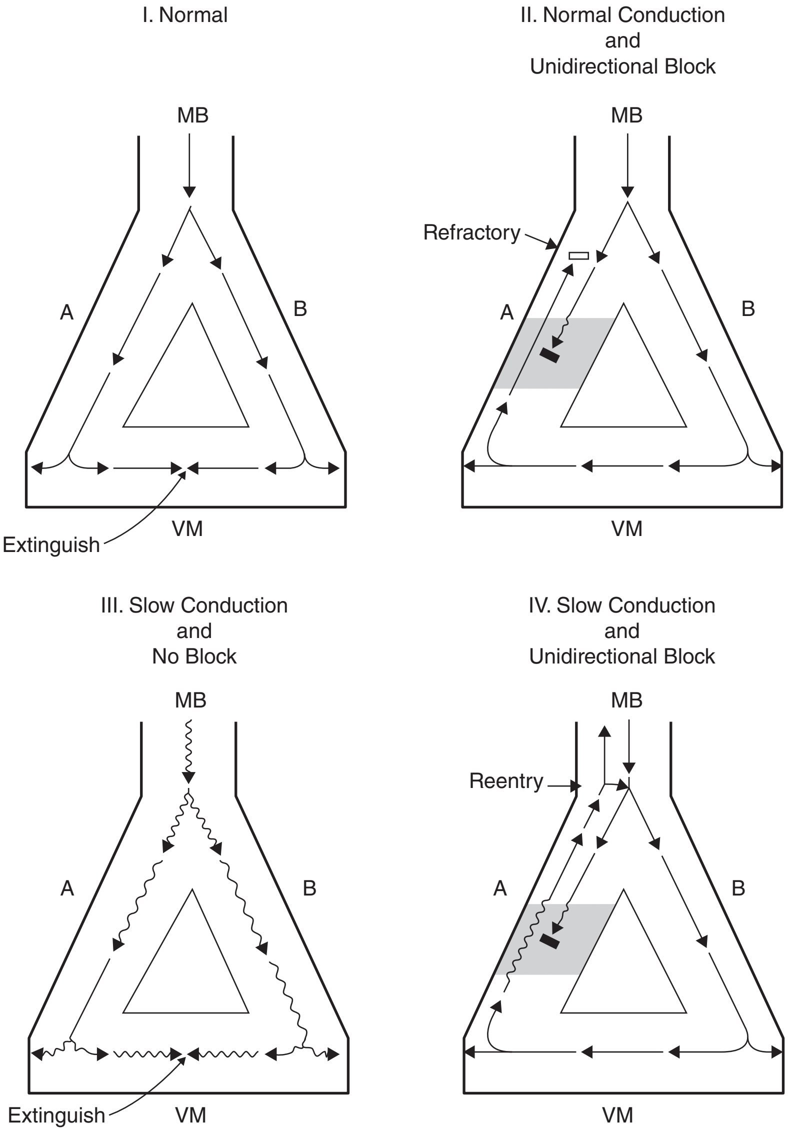
I. Normal
MB
A
B
Extinguish
VM
II. Normal Conduction and Unidirectional Block
MB
Refractory
A
B
VM
III. Slow Conduction and No Block
MB
A
B
Extinguish
VM
IV. Slow Conduction and Unidirectional Block
MB
Reentry
A
B
VM

Figure 22.4 Schematic representations of potential mechanisms involved in cardiac arrhythmias caused by reentry phenomena. I. Normal. The cardiac impulse (arrows) exits a main bundle branch (MB) of the Purkinje system and enters terminal Purkinje branches A and B. The impulse uniformly and rapidly excites a segment of ventricular muscle (VM) and would be extinguished within the VM due to refractoriness of the cells just excited. II. Normal conduction and unidirectional block. Because of an area of damaged tissue (shaded area) that blocks antegrade conduction in branch A, the impulse traversing branch B and the VM will excite branch A. The impulse will traverse branch A and the area of unidirectional block through a retrograde pathway; however, since it is conducted at a normally fast speed, it will encounter refractory cells (open square) and be extinguished. III. Slow conduction velocity and no block. Although the cardiac impulse may be conducted at an abnormally slow velocity (wavy arrows), the lack of unidirectional conduction block causes the impulse to arrive at refractory cells and extinguish. IV. Slow conduction and unidirectional block. Same as II but the speed of impulse conduction through the area of unidirectional block (wavy arrows), and perhaps through B and VM as well, is so slow that the impulse encounters cells after their refractory period. Thus the impulse can reenter the conduction pathway, thereby establishing perpetual reexcitation. Modeled after Cranefield, 1973; Mason et al., 1973. Source: Adams, 1986.

c in I of Figure 22.3), drugs can depress ectopic foci and restore normal sinus rhythm without affecting MDP or TP. If a drug raises TP to less negative values (e.g., from TP-a to TP-b in II of Figure 22.3), additional time will be required to reach TP, thereby depressing automaticity. By increasing the MDP (e.g., from MDP-a to MDP-b in III of Figure 22.3), a drug can suppress automaticity because additional time would be required before TP is attained.

Disturbances in Impulse Conduction

Arrhythmias caused by disturbances in impulse conduction are thought to be associated with a phenomenon of reentry or circus movement. The concept of reentry is based on very slow conduction velocity, an area of the heart demonstrating unidirectional block of impulse conduction and perhaps an abnormally brief refractory period (Schmidt and Erlanger, 1929; Wit et al., 1972, 1974). This theory holds that a cardiac impulse can travel circuitously around an anatomic loop of fibers in which slowed conduction velocity and brief refractoriness permit the impulse to arrive at cells that are no longer refractory, thereby permitting perpetual reexcitation.

A schematic demonstration of impulse reentry at a junctional region between PF and ventricular muscle is shown in Figure 22.4 (Adams, 1986). Conduction can be significantly slowed by pathological lesions in the heart tissue. Because of slowed conduction, a reentry stimulus can excite tissue that would have otherwise been refractory. Reentry theoretically could be controlled by a drug that either creates bidirectional block or bidirectional conduction through the region of cells causing the unidirectional block; accelerates speed of impulse conduction, thus returning the impulse to the site of reentry when cells are still refractory; prolongs action potential duration of normal cells, thereby extending their refractory period; or exhibits a combination of the above actions.

Other forms of cardiac electrophysiological disturbances, in addition to primary abnormalities of impulse conduction and automaticity, may be important. Examples include abnormal excitability, early/late afterdepolarizations, and triggered electrical activities. These types of abnormalities overlap mechanistically with disturbances of automaticity and impulse conduction. Arrhythmias arising from primary automaticity and conduction abnormalities are adequate for modeling the classes of antiarrhythmic drugs relative to their effects on cardiac action potential characteristics and arrhythmogenesis.

Antiarrhythmic Drugs

Based on mechanisms described above, particularly with respect to ion conduction across cardiac cell membranes, antiarrhythimic drugs can be classified using the traditional Vaughn Williams classification scheme discussed in Section Electrophysiological Properties of Cardiac Cells. This classification is presented in Table 22.1.

Antiarrhythmic drugs are divided into Classes I to IV in this system. Although the clinical importance of this classification scheme has been debated (see Vaughn Williams, 1992), some familiarity with the system is still of value. Some drugs (e.g., digoxin, anticholinergic agents, vagolytics, sympathomimetics) are not classified by this system.

Class I antiarrhythmics block fast sodium channels in the myocardial cell membrane resulting in a decrease in the upstroke velocity of the action potential in atrial and ventricular myocardium and Purkinje cells. By decreasing the upstroke velocity, class I antiarrhythmics slow conduction velocity in normal and abnormal cardiac tissue. Class I drugs also prolong the refractory period and can be effective at abolishing reentrant tachyarrhythmias. Class I agents have variable effects on repolarization. Some of them prolong repolarization while others shorten it or have no effect. Class I agents are also subdivided into classes IA, IB, and IC based on differences in electrophysiological and antiarrhythmic differences (Keefe et al., 1981).

Class IA antiarrhythmics depress conduction in normal and abnormal cardiac tissue and prolong repolarization. Examples used in veterinary medicine are procainamide and quinidine.

Class IB antiarrhythmics have a greater impact on conduction velocity and effective refractory period in abnormal cardiac tissue than in normal tissue. Additionally, their effect is most profound in Purkinje fibers and they have very little effect on the sinus node, atrioventricular node, or cardiac contractility. Examples used in veterinary medicine are lidocaine and mexiletine.

Class IC antiarrhythmics include encainide and flecainide. They are rarely, if ever, used in veterinary medicine. Flecanide was withdrawn from the market after adverse effects were reported in people.

Class II antiarrhythmics are the β-adrenergic receptor blockers. These include atenolol, propranolol, and sotalol. Sotalol is also considered a Class III due to its unique combination of beta-blocking and potassium channel blocking mechanisms.

Table 22.1 Classification of antiarrhythmic drugs based on mechanism of action: Vaughn Williams classification system. Source: Data from Vaughn Williams, 1984.

Class	Drug	Effect on Na$^+$ channels	Effect on K$^+$ channels	Effect on Ca^{++} channels	Effect on action potential
Class Ia	Local anesthetics Procainamide Quinidine	Depresses	Depresses	–	Lengthens action potential Increases refractory period
Class Ib	Local anesthetics Lidocaine Mexiletine	Depresses	Depresses	–	Does not increase refractory period as Class Ia drugs
Class II	β_1-adrenergic blockers Propranolol Atenolol Metoprolol	Depresses	–	Depresses	Suppresses SA, AV node Decreases rate
Class III	Potassium channel blockers Amiodarone Sotalol[a]	–	Depresses	–	Prolongs refractory period Lengthens action potential
Class IV	Calcium-channel blockers Verapamil Diltiazem	–	–	Depresses	Suppresses SA, AV node Decreases rate

[a] Sotalol also has beta-blocking (Class II) properties.

Table 22.2 Dose recommendations for drugs used to treat cardiac arrhythmias

Amiodarone	Dogs: 8–10 mg/kg, orally, twice daily be given for one week and then reduced to 5–10 mg/kg PO once daily thereafter
Atenolol	Dogs: 0.25–1.0 mg/kg, orally, twice daily Cats: 6.25–12.5 mg/dog, orally, twice daily
Atropine sulfate	Dogs and cats: 0.02–0.04 mg/kg, subcutaneously every 4–6 hours, or as a single injection for atropine challenge
Digoxin	Dogs: 0.005 mg/kg, orally, twice daily Cats: 0.008–0.01 mg/kg orally, every 48 hours
Diltiazem	Dogs: 0.5–1.5 mg/kg, orally every 8 hours Cats: 1.75–2.4 mg/kg, orally every 8 hours
Esmolol	0.05–0.1 mg/kg intravenously given slowly
Isoproterenol	1 mg diluted in 500 ml of 5% dextrose of Ringer's solution and infuse intravenously at 0.5–1 ml/min or to effect
Lidocaine	Dogs: 2–4 mg/kg, intravenously Cats: 0.25–0.75 mg/kg, intravenously
Mexiletine	Dogs: 5–7 mg/kg, orally, every 8 hours.
Procainamide	Dogs: 10–30 mg/kg, orally, every 6 hours Cats: 3–8 mg/kg, orally, every 6 hours
Propantheline	0.25–0.5 mg/kg, orally, every 8–12 hours
Quinidine	Horses: 22 mg/kg, via nasogastric tube, every 2 hours until conversion to sinus rhythm, a cumulative dose of 88–132 mg/kg had been administered
Sotalol	Dogs: 1–2 mg/kg, orally, every 12 hours Cats: 1–2 mg/kg, orally, every 12 hours
Terbutaline	Dogs: 1.25–5 mg/dog, orally, every 8 hours Cats: 0.1–0.2 mg/kg, orally, every 12 hours

Class III antiarrhythmics prolong the action potential and increase the refractory period. Examples used in veterinary medicine are amiodarone and sotalol.

Class IV antiarrhythmics are the calcium channel blockers. The most common example is diltiazem.

A practical approach to consider which antiarrhythmic to administer is to begin by diagnosing the rhythm abnormality and selecting a drug based on the underlying heart rate or arrhythmia. Initial therapy should be directed at correcting specific etiologies; for example if serum electrolyte abnormalities are responsible, obviously these should be corrected before an antiarrhythmic drug is introduced. Dose recommendations are provided in Table 22.2.

Bradyarrhythmias

Bradyarrhythmias are arrhythmias with heart rates that are below the normal range. Sinus bradycardia may be due to systemic issues including hypothermia, hypothyroidism, and issues that lead to high vagal tone. Sick sinus syndrome and atrioventricular block are bradyarrhythmias that are typically a result of sinus node or atrioventricular node issues. These arrhythmias may be responsive to drugs that can increase heart rate, termed positive chronotropic drugs. However, if they are not responsive to medical therapy, a pacemaker may be needed.

Two mechanisms for increasing heart rate are to decrease vagal tone (vagolytics) or increase sympathetic tone by stimulation of the β-adrenergic receptors in the sinus or atrioventricular nodes (sympathomimetic adrenergic drugs).

Vagolytics such as atropine and propantheline bromide are commonly used to treat bradyarrhythmias that are a result of high vagal tone. These drugs were introduced in Chapter 8. Atropine is frequently used acutely in anesthetized patients in which a bradycardia is observed. It can also be used to determine how responsive a bradyarrhythmia might be to a vagolytic treatment by doing an atropine challenge. In these cases, atropine may be given at a dose of at 0.4 mg/kg subcutaneously and the heart rate rechecked in 30 minutes. An increase in heart rate after the atropine challenge indicates that the arrhythmia may have a component of high vagal tone and may be responsive to an oral vagolytic or sympathomimetic.

Propantheline Bromide

Mechanism of action and pharmacology: Propantheline bromide is an antimuscarinic agent with similar actions to atropine, but it exists in an oral formulation and may be appropriate for chronic use. Propantheline is primarily metabolized in the gastrointestinal tract and liver and is not completely absorbed after oral administration. There is likely a variable rate of absorption among dogs. Because of the lack of specific information regarding oral absorption and onset of action, it is recommended that each case be closely monitored and doses adjusted as needed.

Side effects, adverse effects, and tolerance: As a vagolytic, propantheline could be expected to lead to sinus tachycardia, increased salivation, and vomiting.

Clinical use: In small animal medicine, propantheline can be used an oral treatment for atropine challenge responsive bradyarrhythmias including sick sinus syndrome and possibly second-degree atrioventricular block. However, it may not be as effective as the sympathomimetics described below and is not used as commonly.

Clinical monitoring: Propantheline is used most commonly to increase heart rate in dogs with bradycardias that are responsive to atropine, and response to therapy is variable (Rishniw and Thomas, 2000). Heart rate and rhythm should be monitored with electrocardiography to monitor for a response to therapy. Owners should be advised of possible clinical signs including increased salivation and vomiting.

Isoproterenol

Mechanism of action and pharmacology: Isoproterenol is a sympathomimetic, introduced in Chapter 7, that increases heart rate by stimulating both the β_1 and β_2 receptors in the sinus and atrioventricular node. Although an oral form is available, isoproterenol is most commonly used intravenously for short-term treatment. The intravenous form is very rapid acting and the effect is decreased rapidly after discontinuation.

Side effects, adverse effects, and tolerance: As a stimulant of the β receptors, isoproterenol can lead to tachycardia, increased contractility, hypotension, and arrhythmias, particularly if underlying heart disease is present. However, the side effects will be rapidly reduced after decreasing the dose or by discontinuing the infusion. Although chronic administration is unlikely, long-term exposure can induce congestive heart failure in dogs.

Clinical use: Isoproterenol can be given intravenously for patients with emergency bradycardias (atrioventricular block, sick sinus syndrome) while waiting for surgical intervention with a pacemaker.

Clinical monitoring: Isoproterenol can lead to hypotension as well as cardiac arrhythmias; therefore, blood pressure monitoring and continuous electrocardiography should be performed for the duration of infusion. This also helps determine if the dose should be adjusted for an improved heart rate response.

Tachyarrhythmias

Sinus Tachycardia

Sinus tachycardia is typically a response to low blood pressure, pain, sepsis, fever, or low cardiac output. Note that many of these are systemic issues rather than a primary cardiac issue and do not require specific antiarrhythmic drug treatment. Sinus tachycardia can also be associated with congestive heart failure as a response to low cardiac output. Because sinus tachycardia is most commonly a sign of underlying systemic or cardiac disease, the primary cause should be addressed by correcting underlying problems. Treatments include administration of fluids if needed to correct blood pressure, considering pain relief, and if the sinus tachycardia is secondary to low cardiac output and heart disease, treating the heart disease with cardiac drugs including pimobenden and angiotensin-converting enzyme inhibitors (ACE-inhibitors) as indicated and fully discussed in Chapter 21.

Supraventricular Tachyarrhythmias

In small animals, supraventricular tachyarrhythmias are usually caused by primary cardiac disease and secondary atrial enlargement, and are not likely to completely resolve with antiarrhythmic drug treatment. Therefore, treatment is directed at slowing the rapid conduction through the atrioventricular node with one of three options: digitalis, a calcium channel blocker or a β-adrenergic receptor blocker.

Digoxin

Mechanism of action and pharmacology: Digoxin's mechanism as an antiarrhythmic is predominantly due to a parasympathetic effect (vagomimetic effect) on the sinus node, atrioventricular node and atrial tissue. This class of drugs has been extensively discussed in Chapter 21. This impact slows conduction through the atrioventricular node and increases vagal tone to the ventricle (Ettinger, 2010). The pharmacokinetics have been described in several veterinary species, which has defined the distribution, elimination, and recommendations for dosage regimens. The dose of digoxin is based on the animal's lean body weight because it is preferentially distributed to muscle instead of fat. It is metabolized by the liver and excreted by the kidney. Dose adjustments based on clinical monitoring should be done in patients with both cardiac and kidney disease.

Adverse effects, adverse effects, and tolerance: The most common clinical signs are gastrointestinal and include nausea, loss of appetite, vomiting, and diarrhea. Cardiac arrhythmias may also develop including atrioventricular block, ventricular premature complexes, and tachycardia. Hypokalemia can exacerbate the toxicity.

Clinical use: The most common antiarrhythmic use of digoxin is for the treatment of supraventricular tachycardia by slowing conduction through the atrioventricular node and slowing the ventricular response rate. However, in many cases, the reduction in heart rate is not significant to have clinical importance and it may be necessary to add on a Class IV (calcium channel blocker), typically diltiazem, or a Class II (β-adrenergic receptor blocker), typically atenolol (Gelzer et al., 2009). In cases with congestive heart failure or significant myocardial dysfunction, it may be reasonable to first give digoxin to decrease the heart rate since it is also a positive inotrope (diltiazem and atenolol are negative inotropes). Once the heart disease is more stable, atenolol or diltiazem may be added on to improve the heart rate reduction and to obtain a target rate closer to 150 beats per minute.

Clinical monitoring: As mentioned in Chapter 21, there is tremendous patient variability for digoxin toxicity and individual patient factors including obesity, ascites, hypoalbunimea, renal disease, and thyroid status can all be involved (Merrett, 2000). Due to the difficulty in predicting digoxin toxicity, it is prudent to measure serum levels 10–14 days after starting digoxin. Samples should ideally be drawn 6–8 hours postadministration. Most clinicians aim for a target range of 0.8 – 1.2 ng/ml for dogs (Trepanier, 2013), although some dogs may show clinical signs of toxicity even within the therapeutic range and others will not act at all toxic even when their level is above this range. There is anecdotal evidence that management of atrial fibrillation requires higher concentrations than for treatment of heart failure, but this has not been confirmed through clinical trials. If signs of toxicity develop, digoxin should be completely discontinued for 48 hours before restarting at half of the previous dose. Cats can be particularly sensitive to digoxin and should be started at the low end of the dose. Fortunately, cats infrequently develop significant supraventricular tachycardia and the use of digoxin for this purpose in the cat is relatively uncommon. Digoxin is also used in horses but most commonly in combination with quinidine for the treatment of atrial fibrillation. Since individual animals have such variation in the likelihood of the development of toxicity, owners should always be advised of possible clinical signs of toxicity so that they can watch them if they develop before serum levels are measured.

Calcium Channel Blockers

Mechanism of action and pharmacology: In veterinary medicine, both verapamil and diltiazem are calcium channel blockers (Class IV agents, Table 22.1) that have been used for their antiarrhythmic activity. However, diltiazem is used more commonly due to a much lower level of effects. Diltiazem is an L-type calcium channel blocker that works by inhibiting the transmembrane influx of extracellular calcium ions into myocardial cells and vascular smooth muscle without altering serum calcium concentrations (Tidholm, 2010). It slows the sinus node depolarization rate as well as atrioventricular node conduction and depolarization rate. It can have a use-dependent rate and therefore has a more profound ability to decrease the ventricular rate with faster heart rates (Pariaut, 2014).

Side effects, adverse effects, and tolerance: Adverse effects of diltiazem are fairly rare in the dog although bradycardia and atrioventricular block could develop. In cats, gastrointestinal signs, including vomiting and anorexia have been observed, but are fairly uncommon. Diltiazem is a negative inotrope by virtue of the Ca^{++} channel blockade. This action could potentially produce cardiac decompensation if administered to animals with significant myocardial dysfunction. However, this is uncommon.

Clinical use: As an antiarrhythmic, the most commonly used calcium channel blocker for veterinary medicine is diltiazem and it is used most commonly to treat supraventricular tachycardia.

Clinical monitoring: Diltiazem is used primarily to slow the ventricular response rate in supraventricular tachycardia so assessment of heart rate is important to determine if the effective dose is being provided. An in-house electrocardiogram for 3–5 minutes is an inexpensive way to occasionally monitor the heart rate and rhythm, but it may not give an accurate representation of the resting heart rate when the animal is relaxed in a home setting. Therefore, many clinicians prefer to monitor heart rate control with a 24-hour Holter monitor after 10–14 days of treatment.

Beta-Adrenergic Receptor Blockers

Beta-adrenergic receptor blockers (beta-blocker, Class II agents, Table 22.1) are predominantly used as an antiarrhythmic for supraventricular tachycardias, although they may be helpful in some cases for treating ventricular tachyarrhythmias particularly if sympathetic tone is involved. Additionally, sotalol, a combination beta-blocker and potassium channel blocker (Class II and Class III agent, Table 22.1) is a very effective ventricular antiarrhythmic.

For treatment of supraventricular arrhythmias, beta-blockers are most commonly used to alter the electrophysiological properties of the atrioventricular node and to decrease the ventricular response rate. Additionally they can prolong the time that the atrioventricular junction is refractory to further stimulation, which is particularly helpful to disrupt reentrant circuits that use the atrioventricular node as part of the reentry circuit.

Beta-blockers are classified according to the β-adrenergic receptors that they block (see Chapters 6 and 7). Beta-blockers can selectively block the β_1 receptors, or nonselectively block both β_1 and β_2 receptors, or partially block β_1 receptors. The most commonly used selective beta-blocker for supraventricular tachycardia in veterinary medicine is atenolol. Esmolol, also a selective β_1-blocker, has a very short half-life and can be given intravenously to disrupt very rapid supraventricular tachycardias. Sotalol is a combination beta-blocker and potassium channel blocker that can be used effectively for both supraventricular and ventricular tachycardias. A few other beta-blockers are used in veterinary medicine for their negative inotropic effect or for cardioprotective strategies, including propranolol, metoprolol, and carvedilol. These are much less commonly used as antiarrhythmics.

Atenolol

Pharmacology: Atenolol is a specific β1-adrenergic blocking drug.

Side effects, adverse effects, and tolerance: Very high doses of atenolol can produce bradycardia although this is uncommonly observed. Due to its β-receptor blocking effects, atenolol is a negative inotrope and could exacerbate heart failure or myocardial dysfunction in patients with low cardiac reserve. It should be administered cautiously when these issues are present.

Clinical use: Atenolol is most commonly used in conjunction with digoxin to slow the heart rate in patients with atrial fibrillation or other rapid supraventricular tachycardias.

Clinical monitoring: As for diltiazem, ideally a Holter monitor would be used to monitor heart rate control after 10–14 days of treatment.

Esmolol

Pharmacology: Esmolol is an ultrashort-acting (half-life <10 minutes) β_1 blocker used commonly for intravenous administration. Steady state β-blockade is produced within 10–20 minutes after starting intravenous administration of esmolol in dogs. After discontinuation of drug administration, no detectable β-blockade is apparent at 20 minutes postinfusion, regardless of the dose administered.

Side effects, adverse effects, and tolerance: Esmolol can cause sinus bradycardia and decrease myocardial function so it should be used with care in patients with congestive heart failure or underlying systolic function. Additionally, it can sometimes stop the tachyarrhythmia quite abruptly leading to sinus arrest so careful monitoring is warranted while infusing.

Clinical use: As an antiarrhythmic, esmolol is primarily used for acute termination of very rapid supraventricular tachycardias.

Clinical monitoring: A continuous electrocardiogram should be monitored while infusing esmolol to observe for the onset of bradycardia or acute discontinuation of the supraventricular arrhythmia. An advantage of esmolol is that the half-life is very short; therefore, if adverse effects are observed, termination of the infusion will ordinarily result in attenuation of effects quickly. In cases of the abrupt occurrence of sinus arrest, sometimes epinephrine is needed to increase the sinus rate.

Sotalol

Mechanism of action and pharmacology: Sotalol is a nonselective beta-blocker and potassium channel blocker (Class II and Class III; Table 22.1).

Side effects, adverse effects, and tolerance: As for any beta-blocker, the heart rate is expected to decrease with sotalol administration. It also prolongs the AV nodal refractory period and the PR interval because of its beta-blocking effect and can sometimes produce mild atrioventricular block. Because it is a beta-blocker, a decrease in myocardial contractility is expected but it should not produce adverse effects in patients with normal heart function. Additionally, as an antiarrhythmic, sotalol does have a potential to be proarrhythmic although this would appear to be fairly uncommon in comparison to some of the older antiarrhythmics such as procainamide.

Clinical use: As a beta-blocker and potassium channel blocker sotalol is effective for both supraventricular and ventricular tachyarrhythmias and can be particularly helpful for patients with both types of arrhythmias.

Clinical monitoring: Ideally a 24-hour Holter monitor should be performed after 10–14 days of treatment to evaluate the efficacy and the heart rate control.

Ventricular Tachycardia

The efficacy of an antiarrhythmic on a ventricular tachyarrhythmia likely depends on the mechanism of the arrhythmia. The most likely possible mechanisms for ventricular arrhythmia development include reentry, and triggered and abnormal automaticity. Unfortunately, the mechanism of the arrhythmia cannot be detected from the clinical aspects of a case and it is not possible to select the antiarrhythmic based on a known mechanism for arrhythmia development. Instead, the antiarrhythmic is typically selected from a small number of available antiarrhythmics with tolerable safety profile.

The goals of treating a ventricular tachyarrhythmia are to decrease the number of abnormal ventricular complexes, as well as the complexity of the arrhythmia, and to hopefully reduce clinical signs (generally syncope) and the risk of sudden cardiac death. The most commonly used ventricular antiarrhythmics (sotalol and mexiletine) are effective at reducing the number of ventricular premature complexes (VPCs) and the complexity of the arrhythmia, and some veterinary cardiologists believe that they do decrease episodes of syncope. However, the overall effect of the antiarrhythmics on preventing sudden cardiac death in veterinary medicine is undetermined.

Procainamide

Mechanism of action and pharmacology: Procainamide is a Class IA antiarrhythmic that decreases myocardial excitability, slows conduction velocity, and prolongs refractory period in the atria, ventricles and His-Purkinje system. In humans and other animals, when procainamide is metabolized, an active metabolite is formed, *N*-acetylprocainamide (NAPA), which has moderate antiarrhythmic effects (Class III antiarrhythmic effects). Dogs are the only mammals that do not produce this metabolite (Papich et al., 1986), which may explain why higher concentrations and higher doses are needed to control arrhythmias compared to people.

Side effects, adverse effects, and tolerance: Procainamide can be proarrhythmic. In one canine study, 50% of the dogs showed an increase in VPC number after 14–21 days of oral procainamide (Meurs, 2002). Procainamide can also have mild negative inotropic effects, but this is rarely a clinically significant issue unless the patient has underlying myocardial disease. Other adverse effects can include gastrointestinal signs (vomiting, diarrhea), widening of QRS and QT intervals, and hypotension. Adverse effects are usually dose related. An important adverse effect in people from procainamide is drug-induced lupus. People who are slow metabolizers have a higher incidence of this adverse effect, but it has not been reported in dogs. People are also susceptible to arrhythmias related to long Q-T syndromes, which can be serious (Class III antiarrhythmic effect). Because of these problems in people, the drug is rarely, if ever, used in human medicine and most dosage forms are no longer available.

Clinical use: Procainamide has been used for treatment of ventricular and supraventricular arrhythmias. However, because of serious adverse effects noted above, particularly the proarrhythmia aspect, it is rarely the first choice for either. It can be used to treat very rapid ventricular arrhythmias in which an intravenous route for dosing may be needed, but it should be injected slowly over 10–20 minutes and has a wide dosing range. Therefore, it is usually the second choice for acute treatment of ventricular tachyarrhythmia in comparison to lidocaine (see Section Lidocaine).

Clinical monitoring: Due to the proarrhythmic effect that can be observed, a continuous electrocardiogram should be monitored during infusion of the intravenous form. If the oral formulation is used, a 24-hour Holter monitor should be evaluated after 14–21 days of therapy to make sure that the arrhythmia has improved and not become worse.

Quinidine

Quinidine is now rarely used in small animals. Its primary use in veterinary medicine at this time is for management of atrial fibrillation in the horse.

Mechanism of action and pharmacology: Quinidine is a Class IA antiarrhythmic that decreases the rate of phase 0 depolarization of cardiac cells and also decreases the slope of spontaneous depolarization of Purkinje fibers. Its main benefit for atrial fibrillation is its ability to prolong the effective refractory period of atrial muscle but it also can prolong the effective refractory period of ventricular muscle with relatively less effect on the refractory period of normal pacemaker cells. The capability of quinidine to directly prolong the refractory period of atrial fibers is thought to account for its ability to convert atrial fibrillation to sinus rhythm. However, quinidine also has an atropine-like activity (vagolytic) that leads to improved AV conduction. Therefore, a complication of using quinidine for treating supraventricular tachyarrhythmias is that it can actually result in an increase in ventricular rate before the atrial dysrhythmia itself is controlled. This characteristic seems to be particularly prevalent when quinidine is administered by the intravenous route.

Side effects, adverse effects, and tolerance: The most common side effects of quinidine are cardiovascular and gastrointestinal and can be quite dramatic requiring careful monitoring of horses being treated with the drug. Cardiovascular effects include hypotension, prolongation of the QRS and QT complex, arrhythmias, and decreased contractility. Horses with concurrent systolic dysfunction could advance into congestive heart failure because of effects on myocardial function. Tachyarrhythmias, including both supraventricular and ventricular, and subsequent sudden death have been reported. Quinidine can also lead to urticarial wheals, colic like symptoms, inflammation of the nasal mucosa with respiratory difficulty, and laminitis (Detweiler, 1977). Quinidine is also a well-known inhibitor of P-glycoprotein, a membrane pump that can be important for clearance of some drugs (Fromm et al., 1999). This interaction is attributed to causing the increased (and potentially toxic) concentrations of digoxin when the drugs are administered concurrently. Because of the well-known effects of quinidine on P-glycoprotein, this drug is often considered as a probe to investigate P-glycoprotein-mediated drug interactions.

Clinical use: The primary use of quinidine is for the treatment of atrial fibrillation in horses (Reef et al., 1995). The use in small animals has declined because of lack of oral dosage forms and adverse effects. When used in horses, quinidine is typically administered via nasogastric tube every 2 hours until conversion to sinus rhythm or a cumulative dose of 88–132 mg/kg, or adverse or toxic effects from the drug. Because of the risk of tachyarrhythmias, increased atrioventricular conduction and decreased contractility, digoxin should be administered before quinidine treatment if the horse has evidence of systolic dysfunction, was prone to tachycardia or had a previous history of sustained tachycardia. Digoxin will help slow atrioventricular conduction and control the ventricular rate in atrial fibrillation and flutter. However, care should be exercised in the concomitant use of digoxin and quinidine, since the latter may substantially increase the plasma concentration of the former (Leahey et al., 1978; Fromm et al., 1999). A study has suggested that addition of digoxin to quinidine might improve the atrial fibrillation conversion rate (Lotstra et al., 2015).

Clinical monitoring: The significant clinical signs that can be observed with quinidine lead to a the need for significant monitoring while treating, including blood pressure monitoring, electrocardiography for evidence of arrhythmias or prolongation of the QRS and QT complexes, and measurement of quinidine levels. The detection of significant adverse effects should lead to discontinuation of quinidine and measurement of a plasma quinidine level.

Lidocaine

Lidocaine is perhaps the most common agent administered IV for acute treatment of ventricular arrhythmias.

Mechanism of action and pharmacology: Lidocaine is a Class IB antiarrhythmic that acts to decrease the rate of ventricular firing, action potential duration and absolute refractory period, and increasing relative refractory period. It has very little effect on atrioventricular node or His-Purkinje conduction. It has a very rapid onset (within a few minutes) when given as an intravenous bolus and can last for more than 10 minutes. However, if continued use is indicated, a continuous rate infusion (CRI) can be provided. To avoid the time to reach steady-state concentrations in dogs, a loading dose may be administered (2–4 mg/kg IV) followed by a CRI of 25–75 μg/kg per minute IV. Lidocaine has a very high first-pass metabolism in the liver and is not effective if administered orally. Mexiletine (discussed in Section Mexiletine) is chemically modified to avoid the first-pass effects and can be administered orally.

Side effects, adverse effects, and tolerance: The side effects of lidocaine are most frequently neurological including depression, seizures, and vomiting, particularly at the high end of the dose. Since it is so short acting, the signs usually resolve once the CRI is discontinued. If clinical signs are severe, the infusion should be decreased or discontinued. If seizures develop they can generally be controlled with diazepam but they usually resolve as soon as the lidocaine infusion rate is decreased or discontinued. If further treatment is indicated, it may be worth considering a change to a different intravenous ventricular antiarrhythmic like procainamide. Cats tend to be much more sensitive to the neurological and cardiac effects of lidocaine and its use in this species is not recommended by cardiologists.

Clinical use: Lidocaine is most useful for acutely converting a ventricular arrhythmia to sinus rhythm. Lidocaine is most effective when the serum potassium level of the patient is at the high normal range; therefore, potassium supplementation should be considered for optimal efficacy if the patient is hypokalemic. However, lidocaine is not useful for chronic therapy since it cannot be given orally, so if continued antiarrhythmics are needed, it is necessary to change to an oral antiarrhythmic such as sotalol or mexiletine. Because of the rapid onset and offset of action of intravenous lidocaine, the first and second dose of the oral antiarrhythmic is often given before discontinuing the intravenous lidocaine. This allows the new antiarrhythmic to begin to develop effective serum levels before removing the lidocaine.

If a ventricular arrhythmia is poorly responsive to lidocaine, consider reevaluation of the serum potassium level and possible supplementation since potassium levels should be in the high normal range for maximum lidocaine effect. Additionally, sometimes decreased levels of myocardial magnesium may be associated with ventricular arrhythmias and magnesium supplementation may be helpful. Finally, since the efficacy of an antiarrhythmic is often dependent on the underlying mechanism of the arrhythmia (which is rarely known), it is sometimes prudent to switch to a different antiarrhythmic. If the response to lidocaine appears to be insufficient and the potassium level is appropriate, it may be reasonable to consider switching to intravenous procainamide.

Another clinical use of lidocaine is to treat pain syndromes and intestinal postoperative ileus in horses (Malone et al., 2006). The effect is likely not via a prokinetic action on the intestine (Milligan, et al., 2007), but by improving repair of the intestinal mucosa in the postoperative period. Lidocaine is also included in clinical infusion protocols, often combined with other agents such as ketamine and morphine (“MLK”) to treat perioperative pain.

Clinical monitoring: A continuous electrocardiogram should be observed while treating with lidocaine to observe for efficacy. Additionally, the patient should be observed for neurological signs.

Mexiletine

Mechanism of action and pharmacology: Mexiletine is similar to lidocaine in that it inhibits the inward sodium current and reduces the rate of rise of the action potential. Mexiletine can also interrupt reentry circuits by slowing conduction and depressing membrane responsiveness.

Side effects, adverse effects, and tolerance: Mexilitine can cause similar neurological effects to lidocaine including nausea, anorexia, vomiting, and depression, as well as bradycardia. As with all antiarrhythmics, it can be proarrhythmic but this appears to be quite uncommon. However, overall mexiletine is actually very safe has become a popular and effective ventricular antiarrhythmic. The most common adverse effect noted in animals is gastrointestinal with some mild loss of appetite or vomiting. Cardiologists have observed that giving mexiletine with at least a small meal may alleviate these gastrointestinal effects.

Clinical use: Mexiletine is most useful for chronic management of ventricular arrhythmias and appears to be able to reduce both the number of ventricular premature beats as well as the complexity (runs, bigeminy, etc.) of the arrhythmia. However, in cases that are refractory to management with mexiletine, success is frequently achieved by combination therapy with sotalol. Since mexiletine and sotalol are in different classes of antiarrhythmics they have different mechanisms of action and appear to have a synergistic effect when given together. One of the adverse electrophysiological effects of sotalol is increased action potential duration (Class III effect) and mexiletine may also counteract the adverse effects of sotalol on the action potential duration.

Because it suppresses sodium channels in activated neurons, another use of mexiletine in people – but not reported in animals – is its use for treating chronic pain. It is used to treat pain caused by diabetic neuropathy and nerve injury and much lower doses than the antiarrhythmic dose.

Clinical monitoring: Ideally a 24-hour Holter monitor should be performed after 10–14 days of treatment to monitor for efficacy. The goal would be to have at least an 85% reduction in VPC number and a reduction in complexity (for example, from runs to singles).

Amiodarone

Mechanism of action and pharmacology: Amiodarone is classified as a Class III antiarrhythmic agent (Table 22.1) that prolongs action potential duration and refractory period. It has a high degree of side effects and is typically reserved for use in very difficult to treat arrhythmias that are not responsive to sotalol, mexiletine, or the combination of both. Amiodarone can be used to treat both supraventricular and ventricular tachycardias.

Amiodarone is a complex antiarrhythmic that shows properties of all four of the main classes. It is structurally related to levothyroxine and has high iodine content. It is metabolized to desethylamiodarone in the dog. Desethylamiodarone has important antiarrhythmic effects because of its ability to block fast sodium channels. Amiodarone has unusual pharmacokinetics. After repeated administration, the drug has a long half-life of 3.2 days in the dog. It is very lipophilic and accumulates in adipose tissue up to 300 times the plasma concentration. Once drug administration is discontinued, amiodarone is cleared rapidly from all tissues except adipose tissue. Although it can be given intravenously as well as orally, it is most commonly given orally.

Side effects, adverse effects, and tolerance: Amiodarone can have a number of adverse effects including anorexia and vomiting. Additionally, quite severe cases of neutropenia, thrombocytopenia, and hepatotoxicity have been observed. In many cases, the adverse effects are thought to be dose related and reversible if discontinued early enough, although some deaths have been reported (Kraus et al., 2009). There are reports that Doberman dogs are more prone to adverse effects than other breeds. Alternatively, the adverse effects in dogs may be caused by the drug vehicle, Polysorbate 80, which is known to elicit allergic-like adverse reactions in dogs caused by histamine release. Pretreatment with antihistamines can be considered if these adverse effects are observed.

Clinical use: Amiodarone has been used to treat both atrial fibrillation and ventricular tachyarrhythmias in the dog (Saunders et al., 2006) and atrial fibrillation in horses. However, due to the frequency of significant adverse effects, including hepatotoxicity and neutropenia, which require careful monitoring, amiodarone is often withheld for the treatment of refractory arrhythmias that are not responsive to the safer antiarrhythmics, including sotalol or mexiletine, alone or in combination.

Clinical monitoring: Careful monitoring of liver enzymes, white blood cell and platelet counts should be done on a regular basis with consideration of discontinuing amiodarone if the liver enzymes are elevated or cell counts are down. Amiodarone serum level measurement is available, although how to use this to guide therapy in veterinary medicine is not yet well understood. A 24-hour Holter monitor should be performed after 10–14 days of treatment to monitor for efficacy.

Sotalol

Mechanism of action and pharmacology: As described above in Section Supraventricular Tachyarrhythmias, sotalol is a nonselective, weak beta-blocker and potassium channel blocker (Class II and III; Table 22.1). It is also very effective for treating ventricular tachyarrhythmias.

Side effects, adverse effects, and tolerance: Sotalol can prolong the AV nodal refractory period and the PR interval and can sometimes lead to mild atrioventricular block. Because it is a β-blocker, a decrease in myocardial contractility is expected but is uncommon, particularly in patients with normal heart function. Additionally, as an antiarrhythmic, sotalol does have a potential to be proarrhythmic although this is fairly uncommon.

Clinical use: For ventricular arrhythmias it has been shown to decrease the number and complexity of ventricular arrhythmias as well as syncope (Meurs, 2002). It is a very effective and safe ventricular antiarrhythmic for veterinary medicine. It is frequently given in conjunction with mexiletine for refractory arrhythmias.

Clinical monitoring: Ideally a 24-hour Holter monitor should be performed after 10–14 days of treatment to monitor for efficacy.

Conclusion

Although in many cases cardiac arrhythmias have no clinical importance and do not require treatment, cardiac arrhythmias can lead to the development of significant clinical signs including sudden cardiac death and do sometimes require antiarrhythmic therapy. The decision to treat a cardiac arrhythmia with an antiarrhythmic should be based on many factors including heart rate, type of arrhythmia, presence or absence of clinical signs such as syncope or exercise intolerance, and presence of underlying heart disease. Although there are many antiarrhythmics, only a small number have been well studied and shown to be effective and safe in veterinary medicine. Selection of the antiarrhythmic should be based on the type of arrhythmia, risk of clinical signs, and side effects of the available antiarrhythmics. The perfect antiarrhythmic does not exist and it may be necessary to try more than one, and to consider combinations when appropriate.

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Section 6

Drugs Affecting Renal Function and Fluid–Electrolyte Balance

23

Principles of Acid–Base Balance: Fluid and Electrolyte Therapy, Blood Substitutes

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Composition and Distribution of Body Fluids

Units of Measure

The units of measure commonly utilized in discussion of fluid balance are presented in Table 23.1. Ions or electrolytes combine according to valence (charge) rather than molecular weight. Hence in the case of univalent ions, 1 mM = 1 mEq. One mM of a divalent ion provides 2 mEq. By expressing most electrolyte concentrations in milliequivalents per liter (mEq/l), and comparing the concentration of cations to anions in the body, it becomes clear that electroneutrality exists. Although extracellular cations are often more completely documented in the course of clinical investigations, anions, particularly chloride and bicarbonate, are the electrical counterbalance. Some electrolytes are measured in millimoles per liter (mM/l) because they exist in variable states of protein binding or valence. An example is total calcium, because protein binding confounds any simple assessment of ionized fraction. Phosphorus exists in variable proportions of phosphate and monohydrogen and dihydrogen phosphate, so no valence can be assigned and calculation of milliequivalence is therefore inaccurate. Since mEq/l are the most common and informative unit of comparison for most electrolytes, conversion formulas are also provided in Table 23.1.

Solutes exert an osmotic effect in solution that is dependent only on the number of particles in solution, not on molecular weight or valence. Hence for nondissociable substances, 1 osmole contains 1 mole of substance. If a substance dissociates in solution, the number of osmoles is increased according to the number of particles generated per mole of dissociated substance. For example, each mmol of a completely dissociated NaCl solution yields 2 mOsm. Osmolarity refers to the number of osmoles per liter, and osmolality indicates the number of osmoles per kilogram of solvent (Rose, 1989). In physiological systems the difference between these two is usually small. The concept of osmolality explains why solutions of diverse chemical and electrical composition (e.g., 5% dextrose, 0.9% NaCl, and 1.3% sodium bicarbonate) can all be considered isotonic. For mammals, isotonic solutions equal approximately 300 mOsm.

Body Fluid Compartments

Semipermeable membranes separate most body compartments, allowing the free passage of water and selected solutes. The effective osmolality, or tonicity, of a solution is related to the ability of a solute to attract water and to sustain an increase in osmotic pressure as a result of water movement. For example, two substances with equal ability to attract water down a concentration gradient and across a semipermeable membrane may have very different effects on osmotic pressure, depending upon the movement of the substance itself through the semipermeable membrane. While the measured osmolality of a solution includes all osmoles, whether effective or ineffective, tonicity of a solution relates only to effective osmolality. For example, a solution containing 300 mOsm of nonpenetrating NaCl and 100 mOsm of urea, which can cross plasma membranes, would have a total osmolarity of 400 mOsm and would be hyperosmotic. However, if one put red blood cells in this solution, they would not shrink or swell, because the urea would diffuse into the cells and reach equilibrium inside and outside the cells. Thus, both extracellular and intracellular solutions would have the same osmolarity. There would be no difference in the water concentration across the membrane and no change in cell volume. The solution is therefore considered isotonic.

Ultimately all fluids within the body are in dynamic equilibrium, but it is helpful during fluid therapy to consider body water as existing in several compartments since critical fluid shifts can and do occur. Determination of the volumes of these compartments is problematic, as can be deduced from the large number of different methods that have been used to estimate these volumes (Kohn and DiBartola, 1992). The most common method for assessment of volume in body fluid compartments

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Table 23.1 Units of measure and conversions commonly used in fluid therapy

Term	Abbreviation	Description and conversion
Molecular (formula) weight	MW	Sum of atomic weights of all elements in a chemical formula
Millimole	mmol (mM)	Molecular (formula) weight of a substance in mg, equals 1 mM
Milliequivalent	mEq	Weight, in mg, of an element that combines or replaces 1 mg (1 mmol) of hydrogen (H^+)
Milliosmole	mOsm	Always contains 6.0×10^{23} molecules and equals 1 mmol of a nondissociable substance
Milliequivalent per liter	mEq/l	= mmol/l × valence = [(mg/dl × 10)/MW] × valence

depends upon intravenous administration of a known amount of a dye or radioisotope-tagged substance that distributes only in the compartment of interest. This is followed by assessment of dye or radioisotope concentration in the compartment. Ideally, the indicator substance must distribute rapidly and homogeneously, remain in the space to be measured, not be metabolized or bound, and be nontoxic. The volume of distribution (Vd) of a drug, or in this case a volume marker, may be derived according to the same principles of pharmacokinetics described in Chapter 3.

Total body water (TBW) is approximated at 60% of body weight, but this figure varies from 50 to 75% depending upon age, lean body mass, and individual animal variations. Since fat is lower in water content than lean tissue, obesity is associated with decreased TBW (approximately 50%). To avoid overhydration of obese patients, fluid requirements are best estimated based on lean body mass. Very young animals are about 70–75% water, with TBW declining with advancing age. Table 23.2 provides estimates of selected volumes in dogs. TBW is broadly divided into two types: intracellular (ICF) and extracellular fluid (ECF). The ECF is further divided into four subcompartments: plasma volume, interstitial lymph fluid, transcellular fluid, and fluid present in dense connective tissue and bone. Table 23.3 provides experimentally derived blood volumes as percentages of body weight for various species.

Transcellular fluid is found in diverse locations, including cerebrospinal fluid, pleural cavity, gastrointestinal tract, bladder, synovia, aqueous humor, and peritoneal cavity. Transcellular volumes vary greatly from monogastrics (1–6%) to horses and ruminants (10–15%), dependent largely upon the amount of fluid sequestered in the gastrointestinal tract. Transcellular volumes are not readily mobilized during volume deficits but are of importance in terms of drug disposition and equilibrium. In certain disease processes, transcellular fluids may accumulate, causing ascites, hydropericardium, hydrothorax, synovitis, or other conditions, depending on the location of fluid accumulation.

Fluid and Electrolyte Distribution

Body solutes are not distributed homogeneously throughout TBW. Like drugs, every solute has a defined space or volume of distribution that can be assessed experimentally. As with estimation of body compartment volumes, determination of solute distribution is limited by the features of the labeled solute used. Because

Table 23.2 Approximate volumes of selected fluid compartments in the dog. Source: Data from Kohn and DiBartola, 1992.

Compartment	% Body weight	Method
Total body water (TBW)	60	Indicator substance
Extracellular fluid (ECF)	20–27	Indicator substance
Red blood cells (RBC)	3	Counted + calculations
Plasma volume (PV)	5	Indicator substance
Total blood volume (BV)	5.7–10	Calculated: RBC volume + PV
Interstitial lymph fluid	15	Calculated: ECF − BV
Transcellular fluid	1–6	Estimated
Bone and dense connective tissue	5	Estimated
Intracellular fluid (ICF)	33–40	TBW − ECF

Table 23.3 Approximate values for blood volumes of various animals expressed as percentages of body weight. Values represent averages from approximately 30 references.

Species	Total blood volume	Plasma volume	RBC volume
Dogs	8.5	4.5	4.0
Cats	6.7	4.7	2.0
Chickens	6.5	4.5	2.0
Cattle	5.7	3.8	1.9
Goats	7.0	5.4	1.6
Horses			
Draft	7.0	4.0	3.0
Thoroughbred	10.0	6.0	4.0
Saddle	7.7	5.2	2.5
Pigs	7.5	4.8	2.7
Sheep	6.5	4.5	2.0

Table 23.4 Approximate average concentrations of cations and anions in plasma in normal mammals. Source: Gross, 1994. Reproduced with permission of Springer.

Cations	mEq/l	Anions	mEq/l
Sodium	135–160	Chloride	110–125
Potassium	3–5	Bicarbonate	18–22
Calcium (total calcium 5–10 mM/l)	4–6	Phosphate	1–3[a]
Magnesium	1–3	Sulfate	1–2
Trace elements	1	Lactate	1–2
		Other organic acids	3–5
		Protein	10–16
Total	144–175		144–175

[a] Phosphate exists in variable proportions of phosphate and monohydrogen and dihydrogen phosphate, so no valance can be identified and the number of mEq/l is therefore an estimate (Gross, 1994).

normal vascular endothelium is largely impermeable to formed blood elements and plasma protein, these cells and solutes are usually limited to the plasma. Vascular endothelium is freely permeable to ionic solutes, and the concentration of these ions is almost the same in interstitial as in plasma fluid. Table 23.4 provides estimations of ion composition in plasma of normal mammals.

The volume of ICF and ECF compartments is determined by the number of osmotically active particles in each space. ECF osmolality can be estimated from the following formula (Rose, 1989):

$$\begin{aligned}&\text{ECF osmolality (mOsm/kg)} = 2([\text{Na}^+] + [\text{K}^+])\\&+ \text{glucose}/18 + \text{blood urea nitrogen(BUN)}/2.8\end{aligned}$$

Because cell membranes are permeable to urea and K^+, these substances contribute only ineffective osmoles, as described earlier. At normal blood glucose concentrations, Na^+ is the primary determinant of effective ECF osmolality. Because Na^+ is the most abundant and osmotically active ECF cation, maintenance of an extracellular-to-intracellular sodium gradient is critical and is accomplished by the cell membrane Na^+,K^+-adenosine triphosphatase (ATPase) pump. This pump is also responsible for maintaining appropriate concentrations of intracellular K^+. Because K^+ is the most abundant intracellular cation, the ratio of intracellular-to-extracellular K^+ concentration is the major determinant of the resting cell membrane potential (−70 to −90 mV). Because all body fluid spaces are isotonic with one another, the effective osmolality of the ICF, and indeed TBW, must be equal to that of the ECF. Acute addition or loss of fluid and/or solutes from the body inevitably results in alterations in compartment volumes and tonicity. Homeostatic shifts of fluid between compartments must then occur to return the system to isotonicity.

The critical distribution of water between the plasma and the interstitium is maintained by the colloidal osmotic pressure of plasma protein (oncotic pressure). This is the force that draws water into the capillaries and balances the hydrostatic pressure driving water out. These so-called "Starling forces" describe the capillary balance between forces that favor filtration of water from plasma and those that retain vascular volume:

$$\text{Net filtration (NF)} = K_f[(P_{\text{cap}} - P_{\text{if}}) - (\pi_p - \pi_{\text{if}})]$$

where K_f represents permeability of the capillary wall, P represents hydrostatic pressure in the capillaries (P_{cap}) (blood) or tissues (P_{if}) (interstitial fluid), and π represents oncotic pressure generated by plasma protein (π_p) or filtered proteins and glycosaminoglycans in the interstitium (π_{if}). Applying Starling's relationships yields the prediction that hypoproteinemia (decreased π_p) will increase loss of vascular fluid and that water depletion (with a relative increase in π_p and a decrease in P_{cap}) will promote reabsorption of interstitial fluid into the vasculature (Kohn and DiBartola, 1992). The volume of intracellular water in a given tissue is maintained by intracellular protein. As plasma water decreases, plasma protein competes with intracellular protein for water, resulting in cellular dehydration. Clinical alterations in plasma osmolality may be assessed by comparing measured osmolality in a patient to calculated serum osmolality as determined using Na^+, K^+, glucose, and BUN measurements (see the ECF osmolality equation provided above). Observed changes in the osmolal gap (difference between measured osmolality and the osmolality calculated from normal concentrations) may be useful in determining the presence of unmeasured osmoles associated with toxic substances such as ethylene glycol. The osmolal gap may also be useful in assessing shifts in plasma sodium concentration (Kohn and DiBartola, 1992).

The number of cations in the ECF must equal the number of anions in order to maintain electroneutrality. In practice, only selected cations and anions are routinely measured in a clinical setting. Calculation of the difference between the commonly measured cations and anions in ECF yields the unmeasured anions, or anion gap (Oh and Carrol, 1977; Emmett and Narins, 1977). The anion gap calculation can be useful in assessing the etiology of metabolic acidosis and will be discussed in this context subsequently.

Water, Sodium, and Chloride

Homeostasis

Daily intake of water, nutrients, and minerals is normally balanced by daily excretion of these substances. Water turnover is the term used to describe input and output of body water over a given period of time. Values for water turnover, per 24 hours, in various domestic animals resting in cages or stalls range from about 40 to 132 ml/kg/day. The range is influenced by species, age, and physiological state (Adolph, 1939; Smith, 1970 unpublished data). Extremes of temperature, psychological state, disease, and other variables may change water demands markedly. Water turnover in mature dogs is approximated as 40–60 ml/kg/day, while immature and lactating animals may turn over approximately twice this amount (Muir and DiBartola, 1983). Maintenance fluid needs are defined as the volume of fluid required daily to maintain an animal in zero fluid balance, that is, no net gain or loss of water.

Normal water intake occurs in response to thirst, which is stimulated by plasma hypertonicity and/or contracted ECF volume. Plasma hypertonicity, the primary stimulus, prompts osmoreceptors in the supraoptic and paraventricular nuclei of the hypothalamus to release vasopressin, also called antidiuretic hormone (ADH), which is released into the circulation at the level of the pituitary neurohypophysis. Binding of vasopressin to receptors in the distal nephron and renal collecting duct cells activates adenylyl cyclase and increases intracellular cyclic AMP. A protein kinase cascade initiated by activation of protein kinase A results in opening of luminal water pores in the tubule cell. Permeability of the collecting duct to water and reabsorption of water increase. Sustained release of vasopressin depends additionally upon calcium cycling across the plasma membrane and activation of protein kinase C–dependent pathways. Prostaglandins inhibit the renal response to vasopressin. Drugs with anticyclooxygenase activity that inhibit prostaglandin synthesis thereby enhance the action of endogenous vasopressin. Figure 23.1 summarizes the effects of selected drugs and electrolytes on vasopressin release and action.

If ECF volume and renal perfusion decrease, volume receptors in the renal juxtaglomerular apparatus respond, causing the secretion (or release) of renin, which converts angiotensinogen to angiotensin I. This is the rate-limiting step in the renin–angiotensin system. Angiotensin I is activated to the potent vasoconstrictor angiotensin II in the lung and in endothelial cells throughout the body by angiotensin-converting enzyme (ACE). Angiotensin II stimulates the zona glomerulosa of the adrenal cortex to secrete aldosterone, which, in turn, causes increased reabsorption of sodium from the distal nephron with excretion of K^+ and H^+. Due to the increased concentration of sodium, plasma becomes hypertonic, causing vasopressin release and water retention.

Water intake occurs in response not only to thirst but also to hunger. Water content of food may be as low as 10% (dry food) or as high as 90% (succulent green pasture). Canned pet foods generally contain more than 70% water, and semimoist foods are intermediate (20–40% water) (Lewis and Morris, 1987). Intake of dietary water is governed centrally by appetite control mechanisms rather than by fluid and electrolyte homeostasis. In addition to water intake related to eating and drinking, metabolic water is produced endogenously by catabolism of proteins, fats, and carbohydrates (approximately 5 ml/kg/day) and represents about 10–15% of total water intake in dogs and cats (Anderson, 1983).

Normal water loss occurs via urine, fecal water, and saliva (sensible loss), with insensible losses occurring via evaporation from cutaneous and respiratory epithelia. Insensible losses account for TBW elimination of about 15–30 ml/kg/day in healthy, sedentary animals in a thermoneutral environment (Kohn and DiBartola, 1992).

Metabolic rate, and therefore a portion of daily water turnover, are directly proportional to the ratio of body surface area to total volume. For example, the surface area to volume ratio in a puppy is much larger than in an adult dog and the puppy has a higher basal metabolic rate. Both lead to a much greater evaporative loss of water from the skin per unit volume. Hence, daily water turnover per unit body weight may be nearly twice that of the adult animal. Small, immature animals are therefore at greater risk for insensible water loss than large, mature animals.

The most important and predictable loss of water in healthy, sedentary animals, in a thermoneutral environment, occurs via the urine. Urinary losses can vary from 2 to 20 ml/kg/day. Daily urinary water losses may be divided into obligatory water loss and free water loss (Kohn and DiBartola, 1992). Obligatory water loss represents water eliminated in order to excrete the daily renal solute load. The renal solute load is derived from dietary sources of protein and minerals and consists of urea, Na^+, K^+, Ca^{++}, Mg^{++}, NH_4^+, and other cations, and PO_4^{3-}, Cl^-, SO_4^{2-}, and other anions. Hence, daily renal solute load is a function of the quantity and composition of food ingested. Urea accounts for two-thirds of the urinary solute load in dogs (O'Connor and Potts, 1969).

In normal animals increased urine solute load is eliminated by an increase in urine volume (obligatory water

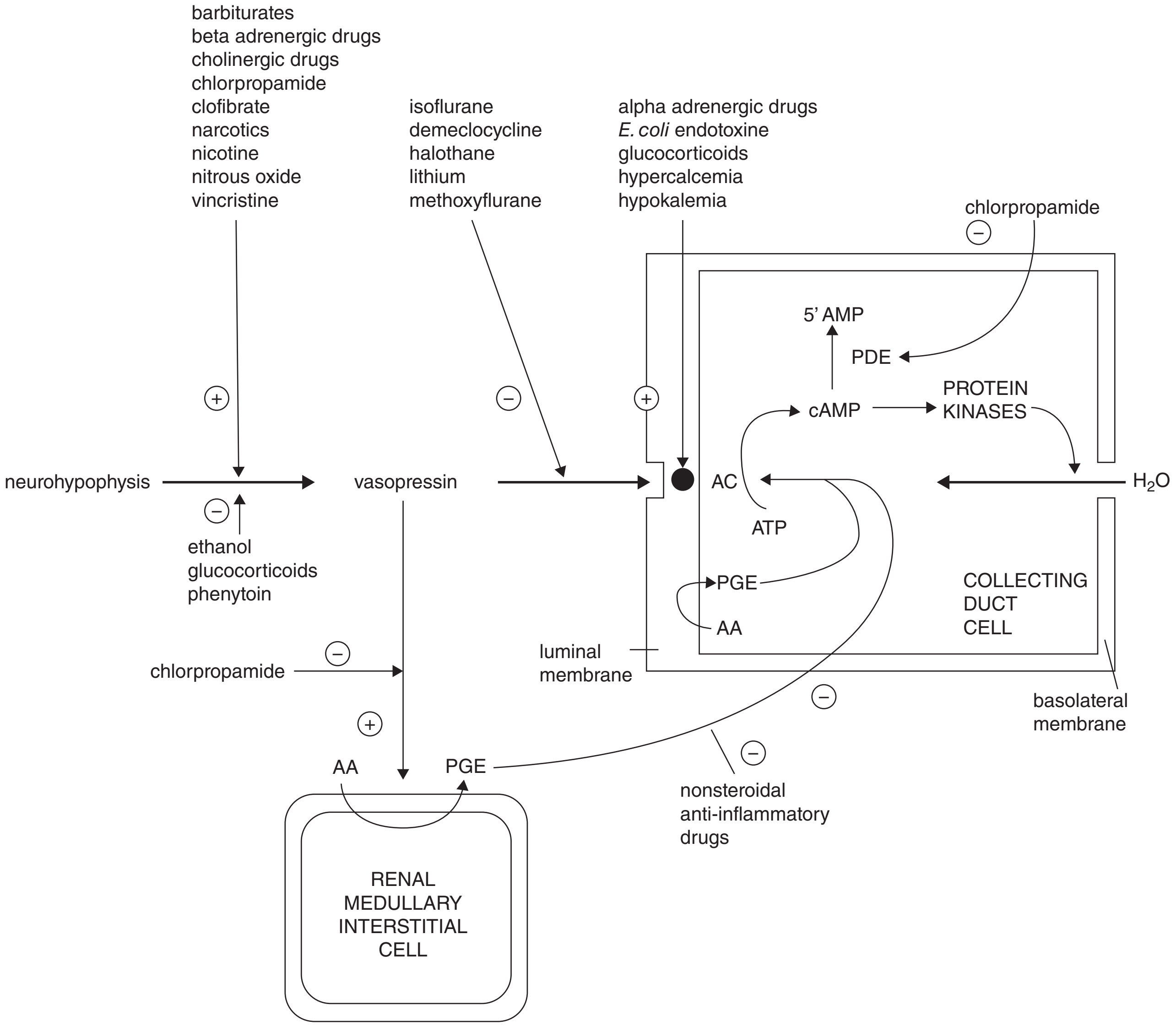


Figure 23.1 Effects of drugs and electrolytes on vasopressin release and mechanisms of cellular action. AA, arachidonic acid; AC, adenylyl cyclase; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; PDE, phosphodiesterase; PGE, prostaglandin E.
Source: Adapted from DiBartola 1992c. Reproduced with permission of Elsevier.

loss) rather than a marked increase in urine osmolality. Hence, urine osmolality is not generally maximized in order to accomplish steady-state elimination of solutes. Obligatory renal water loss is clinically important for removal of renal solutes but also because this type of water loss will continue even in states of relative water deficit. Free water loss represents water excreted unaccompanied by solute. Excretion of free water is controlled by vasopressin and increases during relative water excess or hypotonicity and decreases during water deficit or hypertonicity. Obligatory fecal water loss occurs in order to excrete fecal solutes. Fecal losses ordinarily account for 2–5% of TBW losses and vary with the species. Feces typically contain 50–80% water (Kohn and DiBartola, 1992).

Renal Regulation of Sodium, Chloride, and Water Excretion

Elimination or conservation of body water and solutes via the kidneys depends upon the processes of glomerular filtration and renal tubular reabsorption and secretion. A major mechanism for conservation of water is urine concentration. The canine kidney can concentrate urine to as much as 2400 mOsm, compared to 1200–1400 mOsm achieved in human urine. Elimination of substances via the urine depends upon renal clearance of each substance from the plasma. The volume of plasma that must be filtered each minute to account for the amount of substance appearing in the urine each minute under steady-state conditions defines renal clearance of that substance.

As much as 20% of cardiac output is directed to the kidneys, with blood entering a renal glomerulus through an afferent arteriole and leaving through an efferent arteriole. Resistance changes in afferent and efferent capillaries regulate glomerular filtration rate (GFR). For discussions of normal and abnormal renal physiological function the reader is referred to any standard physiology text. An understanding of the complexities of renal function is crucial to the understanding of water, acid–base, and electrolyte balances.

As glomerular filtrate flows through the tubules, most of the water (greater than 90%) and varying amounts of solute are reabsorbed into the peritubular capillaries. The composition of the tubular reabsorbate closely approximates that of ECF. Reabsorption is largely achieved by transport of electrolytes and other solutes in two steps: (i) absorption of solutes from tubular fluid into tubular cells and (ii) movement of solutes from tubular cells into the ECF. Several types of transport account for tubular reabsorption of solutes, including passive transport (simple diffusion), facilitated diffusion, active transport, and cotransport. These mechanisms are discussed in more detail in the context of diuretic drugs (Chapter 24) and are summarized in Figure 23.2, which depicts some of the functional processes for regulation of salt and water transport in different segments of the nephron.

As much as 60–65% of filtered solute is reabsorbed in the proximal tubule accompanied by osmotically proportional amounts of water. The tubular fluid at the distal portion of the proximal tubule becomes slightly hypoosmotic. Passive reabsorption of substances, especially sodium and chloride, continues in the thin segment of the loop of Henle. The thick ascending limb of the loop of Henle and the distal convoluted tubule are relatively impermeable to water but actively reabsorb solute. Sodium and chloride enter tubular cells in the thick ascending limb of Henle's loop by crossing the luminal membrane coupled to potassium in a proportion of 1 Na^+ : 1 K^+ : 2 Cl^-. Sodium is then actively extruded across the basolateral membrane to maintain intracellular sodium at low levels. Potassium and chloride leave the tubular cell passively. Two consequences of this are decreased concentration of sodium and chloride in the tubular lumen and increased concentration of each in interstitial fluid. A concentration gradient across the tubular epithelium is established, and this becomes multiplied in a longitudinal direction by the countercurrent mechanism. The collecting ducts are responsive to vasopressin, and in its presence the ducts become highly permeable to water. Tubular fluid equilibrates with hyperosmotic interstitium, and hypertonic (concentrated) urine results. In the absence of vasopressin, the

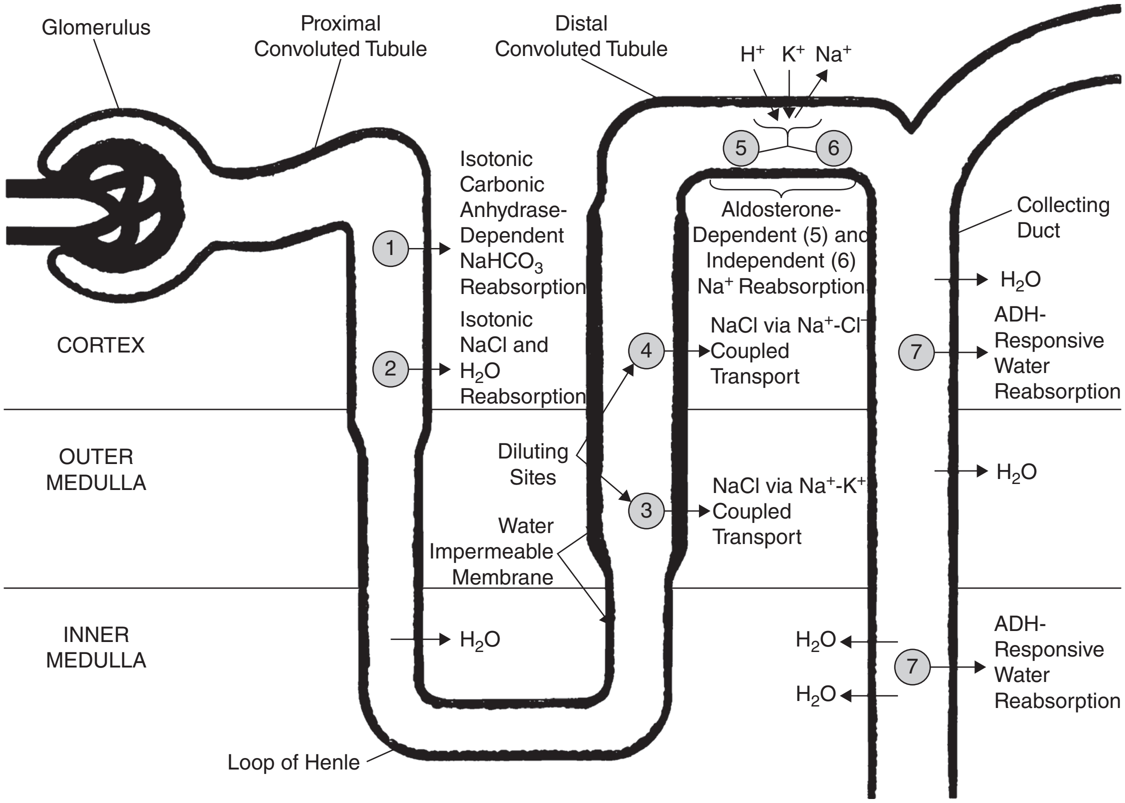

Glomerulus
Proximal Convoluted Tubule
Distal Convoluted Tubule
H+ K+ Na+
5
6
Isotonic Carbonic Anhydrase-Dependent NaHCO3 Reabsorption
1
Aldosterone-Dependent (5) and Independent (6) Na+ Reabsorption
Collecting Duct
H2O
CORTEX
Isotonic NaCl and H2O Reabsorption
2
4
NaCl via Na+-Cl− Coupled Transport
7
ADH-Responsive Water Reabsorption
OUTER MEDULLA
Diluting Sites
H2O
3
NaCl via Na+-K+ Coupled Transport
Water Impermeable Membrane
INNER MEDULLA
H2O
H2O
7
ADH-Responsive Water Reabsorption
H2O
Loop of Henle

Figure 23.2 Functional processes for regulation of salt and water transport in a nephron. Source: Thier 1987. Reproduced with permission of Taylor & Francis.

ducts are relatively impermeable to water. In this case, sodium and chloride have been reabsorbed proximally to the collecting ducts, tubular fluid is hypoosmotic, and voided urine is dilute (Thier, 1987).

Renal reabsorption of sodium in the distal nephron is increased by aldosterone, a mineralocorticoid synthesized in the zona glomerulosa of the adrenal cortex. Aldosterone is produced and released in response to stimulation by angiotensin II, hyperkalemia, and by a decrease in dietary sodium intake. Adrenocorticotropic hormone (ACTH) and hyponatremia play permissive roles in promoting aldosterone secretion. Increased dietary sodium and atrial natriuretic peptide (ANP) decrease aldosterone production. ANP is a polypeptide released from atrial and ventricular myocytes in response to atrial distention associated with volume expansion. ANP causes vascular smooth muscle relaxation, inhibits production of aldosterone in the adrenal glands, and blocks the production of angiotensin II. Study results suggest that parathyroid hormone (PTH) is required for augmented ANP secretion in response to acute volume loading in rats. PTH may play an important role in the regulation of fluid homeostasis via control of ANP (Geiger et al., 1992).

In general, chloride is reabsorbed with sodium throughout the nephron. As previously noted, chloride is exchanged in a ratio of 1 Na^+ : 1 K^+ : 2 Cl^- in the thick ascending limb of Henle's loop during sodium reabsorption. Because the cotransporter in this exchange has a very high affinity for both Na^+ and K^+, luminal Cl^- concentration is normally the rate-limiting step in NaCl entry into the cell (Gregor and Velazquez, 1987). Additional active and passive processes contribute to proximal Cl^- reabsorption in the renal tubules. Chloride exchange for formate appears to occur via an anion exchanger in the luminal membrane. Low concentrations of filtered formate combine with H^+ to form formic acid (HF) in the tubular lumen. Because HF is uncharged, it moves freely into the tubular cell. Two additional mechanisms set the stage for conversion of HF back to formate and H^+. First, basolateral Na^+,K^+-ATPase pumps maintain a low intracellular sodium concentration, and this, in turn, allows for the continued exchange of Na^+-H^+ across the luminal membrane. As Na^+ is reabsorbed and H^+ is secreted, the interior of the cell is left with a lower $[H^+]$ than the tubular lumen. Under these conditions HF is converted back to H^+ and formate, providing for continued chloride–formate exchange. Reabsorbed chloride is returned to the ECF across the basolateral membrane by selective Cl^- channels and a K^+-Cl^- cotransporter (Rose, 1994). Additional transport mechanisms in type B intercalated cells in the cortical collecting tubule may exchange bicarbonate for chloride. The favorable inward concentration gradient for chloride (lumen concentration greater than inside the cell) presumably provides the energy for bicarbonate secretion via this mechanism (Bastani et al., 1991).

Understanding the mechanisms for renal regulation of acid–base balance and electrolyte transport is increasingly dependent upon use of transgenic mice in which the function and regulation of key transporter proteins can be assessed (Cantone et al., 2006).

Disorders of Water, Sodium, and Chloride Balance

Types of Dehydration

Dehydration may be considered in three general categories: hypertonic, isotonic, and hypotonic. Pure water loss and loss of hypotonic fluid lead to hypertonic dehydration. As pure water is lost from the ECF, fluid shifts from the intracellular to the extracellular compartment in response to increased osmolality. The resulting proportionate distribution of volume loss results in fewer clinically detectable signs of volume depletion in the patient. Causes of dehydration associated with pure water deficit include hypodypsia due to neurological disease, diabetes insipidus, respiratory losses during exposure to elevated temperatures, fever, and inadequate access to water.

Loss of hypotonic fluid, as compared to pure water, results in a greater depletion of ECF volume since there is less osmotic drive to pull volume from the intracellular space. Hypotonic fluid losses are common and have been subclassified as extrarenal and renal. Extrarenal losses could include gastrointestinal (e.g., vomiting or diarrhea) or third-space loss (e.g., pancreatitis, peritonitis, as a result of surgery or cutaneous injury). Third spacing is a term used to describe extravasation of fluid from the vascular compartment into extravascular spaces. As tonicity of lost fluid approaches or exceeds normal plasma osmolality (about 300 mOsm/kg), disproportionate depletion of ECF causes more evident clinical signs of dehydration. Volume depletion would likely be the most clinically apparent in cases of hypertonic fluid loss.

Estimations of percent dehydration based on clinical signs are given in Table 23.5. Skin elasticity is a useful indicator of hydration status. However, age of the animal, body condition, and the technique used for evaluating elasticity may affect hydration assessment. With advancing age or cachexia, loss of fat and protein may account for decreased skin elasticity unrelated to hydration. Conversely, obese animals are likely to retain skin elasticity longer in the face of dehydration. Possibly as a result of variations in elastin content of skin, some species display smaller changes in elasticity for a given degree of dehydration. This may be clinically important in the horse. While dry mucous membranes can indicate dehydration, open-mouthed breathing associated with respiratory disease may cause misleading mucous membrane dryness. Degree of enopthalmos is considered a very useful parameter in assessment of dehydration in large animals.

Table 23.5 Physical findings in dehydration

Percent dehydration	Clinical signs
4 or less	History of fluid loss, mucous membranes still moist, evidence of thirst
5–6	Subtle loss of skin elasticity, slight delay in return of skin to normal position, hair coat dull, mucous membranes slightly dry but tongue still moist
7–8	Definite delay in return of skin to normal position, both mucous membranes and tongue may be dry, eyeballs may be soft and sunken, slight prolongation of capillary refill time
9–11	Tented skin does not return to normal position, definite prolongation of capillary refill time, eyes definitely sunken in orbits, all mucous membranes dry, may be signs of shock such as tachycardia, cool extremities, rapid and weak pulses
12–15	Definite signs of shock and circulatory collapse, death is imminent

For example, the measured gap between the eyeball and orbit has been included as a guideline for assessment of dehydration in neonatal calves. A gap less than 0.5 cm is correlated with 9–10% dehydration, and a gap greater than 0.5 cm suggests 11–12% loss of hydration (Naylor, 1996). A study in diarrheic calves evaluated several clinical and laboratory parameters to determine which were most useful in assessment of dehydration. Factors assessed included extent of enophthalmos, skin-tent duration on neck, thorax, and upper and lower eyelids, heart rate, mean central venous pressure, peripheral (extremities) and core temperatures, packed-cell volume, and hemoglobin and plasma protein concentration. The best predictors of degree of dehydration were extent of enophthalmos, skin elasticity on neck and thorax, and plasma protein concentration (Constable et al., 1998). Laboratory parameters such as hematocrit, plasma protein, and osmolality are often useful, but assessment should include consideration of possible preexisting derangements, such as anemia or hypoproteinemia, that could confound interpretation. If an accurate previous body weight is known, serial changes in weight are considered a very useful and accurate measurement in determining degree of dehydration.

Hypernatremia

As the most important and abundant ECF cation, sodium is essential for proper maintenance of membrane potentials, initiation of action potentials, and, according to strong ion difference theory, maintenance of acid–base balance. Plasma sodium concentration and plasma osmolality generally vary in parallel since sodium and its associated anions account for greater than 95% of plasma osmolality. Plasma sodium concentration reflects the ratio of body sodium ion concentration to TBW. Total body sodium content, however, is independent of plasma sodium concentration and may be increased, decreased, or unchanged in the presence of hyper- or hyponatremia.

Clinical signs associated with alterations in serum sodium are more related to the rapidity of change rather than to the magnitude of sodium increase or decrease. Hypernatremia (e.g., >155 mEq sodium/l in dogs) and ECF hypertonicity can be caused by a loss of pure water, a loss of hypotonic fluid (extrarenal or renal), or a gain of impermeable sodium-containing solute (Figure 23.3). Clinical signs of hypernatremia are usually observed in dogs and cats as sodium concentration approaches and exceeds 170 mEq/ml. The signs seen are related to the osmotic movement of water out of cells. Negative effects of cellular dehydration are most pronounced in the brain and lead to the characteristic neurological deficits associated with hypernatremia. These deficits include abnormal behavior and mentation, ataxia, seizures, and coma. The more rapidly water shifts out of brain cells, the greater the chance that decreased brain volume will lead to rupture of cerebral vessels and focal hemorrhage (Arieff and Guisado, 1976). If sodium concentration or concentration of sodium-containing impermeable solute increases slowly, the brain attempts to adapt to the hypertonic state by production of intracellular solutes (e.g., sugars, amino acids) known as “idiogenic” osmoles. Production of these osmotically active substances protects the cell by retaining intracellular volume and preventing cellular dehydration. In addition to neurological deficits, other clinical signs of hypernatremia include thirst, anorexia, lethargy, vomiting, and muscle weakness. If hypernatremia is related to hypotonic fluid loss, then clinical signs of dehydration (as previously described) may be present. If a gain of sodium has caused the hypernatremia, volume overload may be a problem, especially in patients with cardiac disease.

Restoration of ECF volume and tonicity is of primary importance in treatment of hypernatremia. Volume replacement must be accomplished slowly to avoid rapid shifts in plasma osmolality. In general, the rate of fluid administration is determined by the rate of onset of the hypernatremia. When treating chronic hypernatremia, the serum sodium concentration should drop at a rate that does not exceed 0.7 mEq/l/h (O’Brien, 1995). If plasma osmolality drops quickly, water may be attracted intracellularly by idiogenic osmoles, resulting in development of cerebral edema.

Patients with clinical hypernatremia are also often dehydrated, and need volume resuscitation. Custom fluid combinations can be designed to gradually bring down sodium while being able to give high rates of fluids to replace volume. In the case of pure-water loss, volume can be replaced with 5% dextrose in water over a 48- to

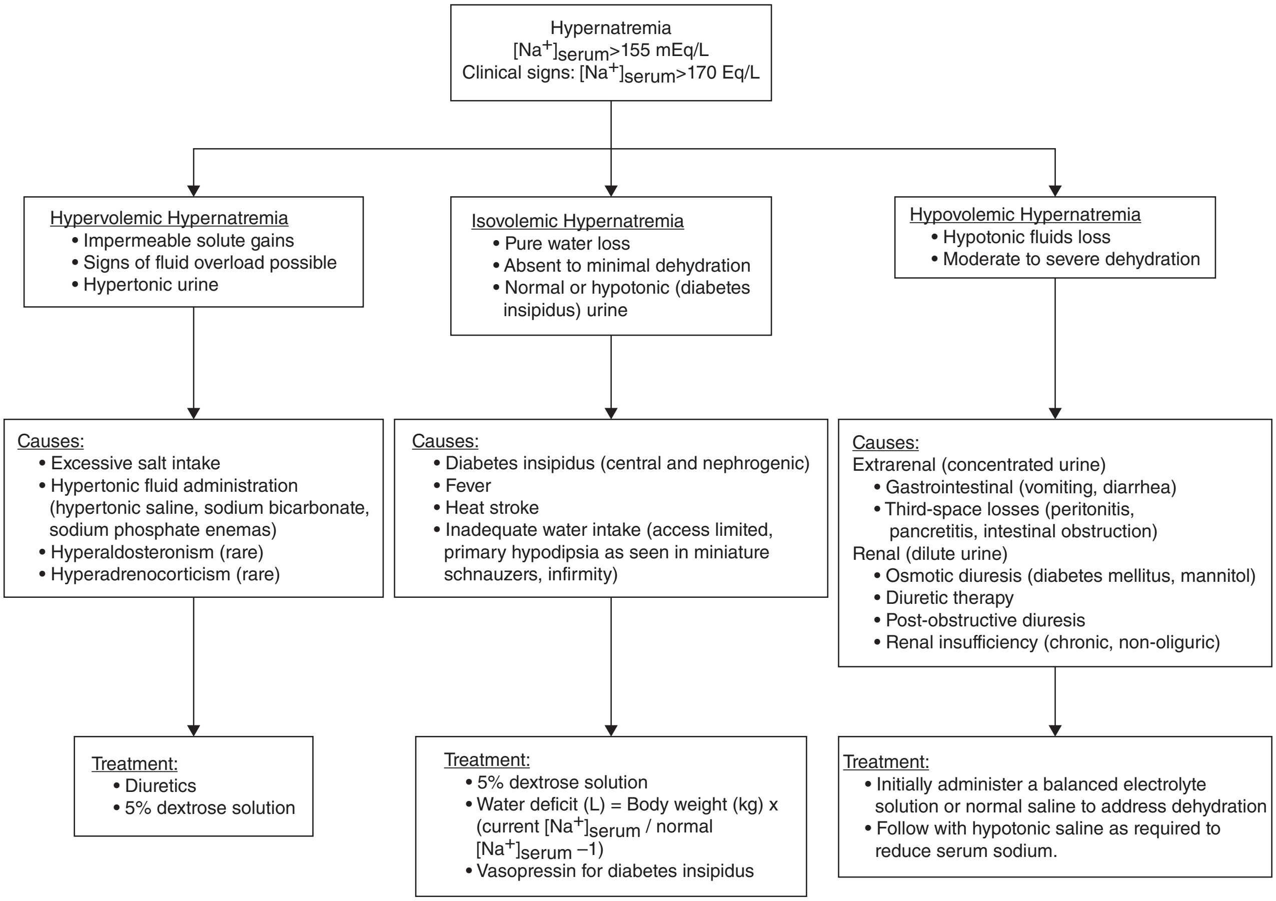

Hypernatremia
[Na+]serum>155 mEq/L
Clinical signs: [Na+]serum>170 Eq/L
Hypervolemic Hypernatremia
• Impermeable solute gains
• Signs of fluid overload possible
• Hypertonic urine
Isovolemic Hypernatremia
• Pure water loss
• Absent to minimal dehydration
• Normal or hypotonic (diabetes insipidus) urine
Hypovolemic Hypernatremia
• Hypotonic fluids loss
• Moderate to severe dehydration
Causes:
• Excessive salt intake
• Hypertonic fluid administration (hypertonic saline, sodium bicarbonate, sodium phosphate enemas)
• Hyperaldosteronism (rare)
• Hyperadrenocorticism (rare)
Causes:
• Diabetes insipidus (central and nephrogenic)
• Fever
• Heat stroke
• Inadequate water intake (access limited, primary hypodipsia as seen in miniature schnauzers, infirmity)
Causes:
Extrarenal (concentrated urine)
• Gastrointestinal (vomiting, diarrhea)
• Third-space losses (peritonitis, pancretitis, intestinal obstruction)
Renal (dilute urine)
• Osmotic diuresis (diabetes mellitus, mannitol)
• Diuretic therapy
• Post-obstructive diuresis
• Renal insufficiency (chronic, non-oliguric)
Treatment:
• Diuretics
• 5% dextrose solution
Treatment:
• 5% dextrose solution
• Water deficit (L) = Body weight (kg) x (current [Na+]serum / normal [Na+]serum −1)
• Vasopressin for diabetes insipidus
Treatment:
• Initially administer a balanced electrolyte solution or normal saline to address dehydration
• Follow with hypotonic saline as required to reduce serum sodium.

Figure 23.3 Summary of classification, causes, and treatment of hypernatremia. See text for additional details of treatment.

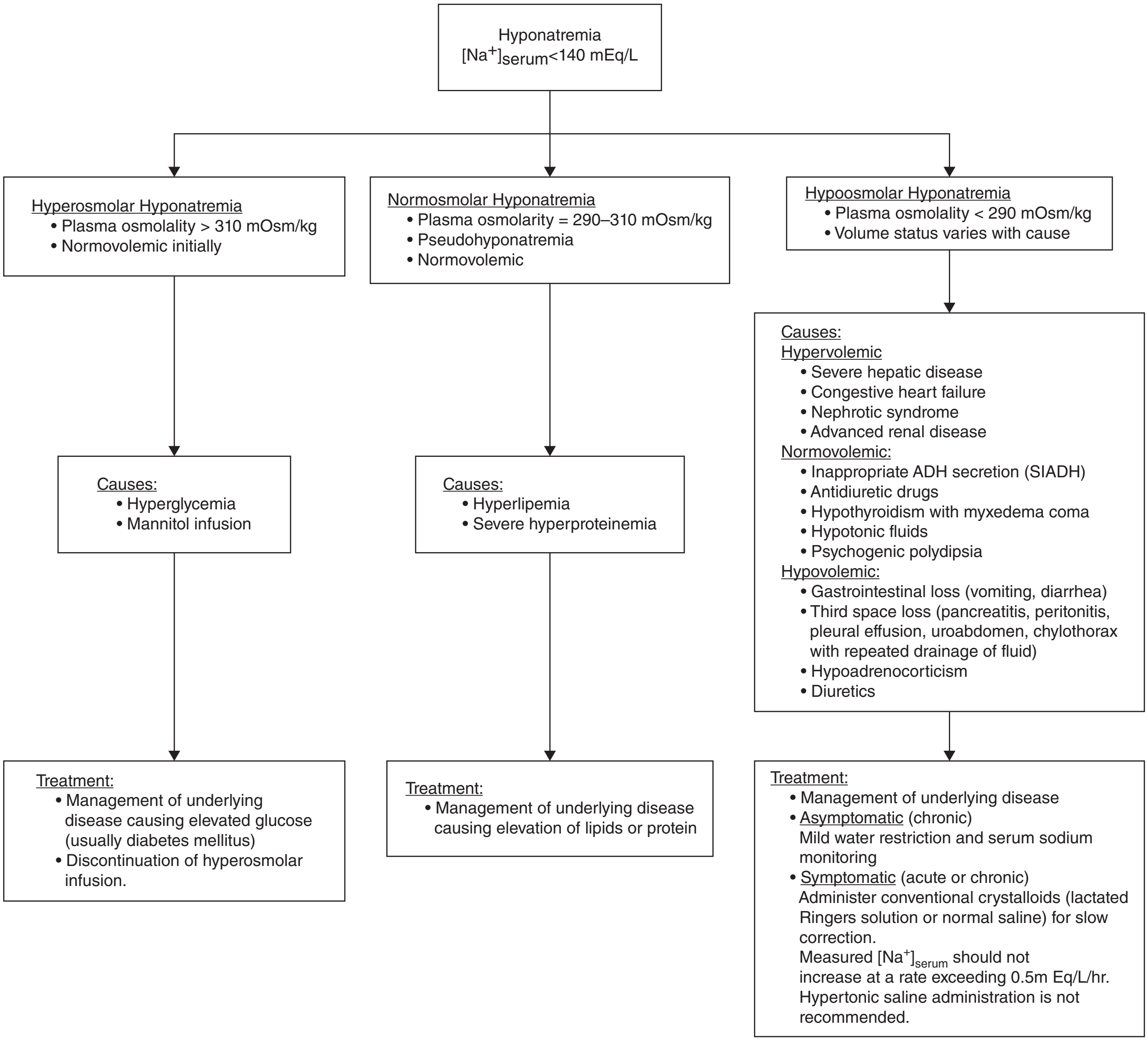
72-hour period. Since the dextrose ultimately enters cells and is metabolized, 5% dextrose administration is essentially replacement with pure water. Use of a 1 : 1 mixture of normal saline with 5% dextrose solution yields an isotonic solution of 2.5% dextrose, 0.45% saline that has also been utilized. This solution decreases plasma tonicity more slowly and decreases the chance for cerebral edema. Hypotonic fluid losses should generally be replaced with an isotonic crystalloid solution. If hypernatremia has resulted from addition of sodium or sodium-containing impermeable solute, then administration of 5% dextrose and water should be accomplished cautiously to avoid pulmonary edema. Diuretics may be useful in promoting saluresis (sodium excretion) as ECF volume is restored (Marks, 1998).

Evaluation and treatment of hypernatremia in critically ill cats was reviewed with emphasis on the importance of careful monitoring and early recognition of signs for positive therapeutic outcomes (Temol et al., 2004).

Hyponatremia

Causes of hyponatremia (<135–140 mEq sodium/l) are best categorized if two additional variables, osmolality and hydration, are also considered. As indicated in Figure 23.4, the more common causes of hyponatremia are accompanied by decreased plasma osmolality (<290 mOsm/kg) with or without volume depletion. If volume depletion exists with hyponatremia, then loss of body sodium has exceeded water loss. Physiological responses to hypovolemia lead to impaired water excretion and a relative dilution of the sodium remaining in body fluids. Hypovolemia causes decreased renal perfusion and GFR, leading to a decline in water excretion. Slower movement of filtrate through renal tubules enhances isosmotic reabsorption of salt and water in the proximal tubules and decreases presentation of tubular fluid at distal diluting sites. Additionally, hypovolemia prompts vasopressin release, further impairing water elimination. Finally, thirst related to hypovolemia results in consumption of low-sodium fluids that also dilute existing plasma sodium (DiBartola, 1992c).

Hyponatremia accompanied by hypervolemia and low plasma osmolality occurs in clinical disorders where there is a physiological perception of volume depletion by in vivo volume detectors. The physiological response



Hyponatremia
$[Na^+]_{serum}$<140 mEq/L

Hyperosmolar Hyponatremia
- Plasma osmolality > 310 mOsm/kg
- Normovolemic initially

Causes:
- Hyperglycemia
- Mannitol infusion

Treatment:
- Management of underlying disease causing elevated glucose (usually diabetes mellitus)
- Discontinuation of hyperosmolar infusion.

Normosmolar Hyponatremia
- Plasma osmolarity = 290–310 mOsm/kg
- Pseudohyponatremia
- Normovolemic

Causes:
- Hyperlipemia
- Severe hyperproteinemia

Treatment:
- Management of underlying disease causing elevation of lipids or protein

Hypoosmolar Hyponatremia
- Plasma osmolality < 290 mOsm/kg
- Volume status varies with cause

Causes:
Hypervolemic
- Severe hepatic disease
- Congestive heart failure
- Nephrotic syndrome
- Advanced renal disease

Normovolemic:
- Inappropriate ADH secretion (SIADH)
- Antidiuretic drugs
- Hypothyroidism with myxedema coma
- Hypotonic fluids
- Psychogenic polydipsia

Hypovolemic:
- Gastrointestinal loss (vomiting, diarrhea)
- Third space loss (pancreatitis, peritonitis, pleural effusion, uroabdomen, chylothorax with repeated drainage of fluid)
- Hypoadrenocorticism
- Diuretics

Treatment:
- Management of underlying disease
- Asymptomatic (chronic)
  Mild water restriction and serum sodium monitoring
- Symptomatic (acute or chronic)
  Administer conventional crystalloids (lactated Ringers solution or normal saline) for slow correction.
  Measured $[Na^+]_{serum}$ should not increase at a rate exceeding 0.5m Eq/L/hr.
  Hypertonic saline administration is not recommended.

Figure 23.4 Summary of classification, causes, and treatment of hyponatremia. See text for additional details of treatment.

is volume expansion. For example, in congestive heart failure, decreased cardiac output is sensed as volume depletion by baroreceptors. Release of vasopressin impairs water excretion, leading to expanded vascular volume. Decreased effective circulating volume and decreased renal perfusion also lead to activation of the renin–angiotensin–aldosterone system. Enhanced renal retention of sodium contributes to expanded vascular volume. In cirrhosis and the nephrotic syndrome, hypoalbuminemia and decreased oncotic pressure may contribute to decreased effective circulating volume and, ultimately, vasopressin release and volume expansion. Other features of hepatic and renal disease also contribute to decreased circulating volume and/or impaired water excretion (DiBartola, 1992c).

Hyponatremia is relatively less common when associated with increased plasma osmolality. The most frequent cause of sodium decreases in the presence of increased plasma osmolality is the increased circulating glucose levels associated with diabetes mellitus. Each 100 mg/dl increase in glucose results in a measured decrease of serum sodium by 1.6 mEq/l (Katz, 1973). In response to the increased concentration of serum glucose, water shifts from the intracellular to the extracellular compartment, resulting in dilution of measured sodium. Serum osmolality remains high due to elevated glucose concentrations. Hyponatremia associated with normal plasma osmolality is referred to as pseudohyponatremia. The decreased sodium concentrations are spurious and are almost universally related to technical difficulties in sodium measurement when plasma lipid or protein concentrations are high.

As with hypernatremia, clinical signs of hyponatremia are more severe if sodium concentration changes rapidly than if it changes over a more prolonged period of time. If sodium concentrations and plasma osmolality decrease quickly, water shifts out of the ECF and into cells. The central nervous system (CNS) is most affected by a rapid fluid shift, which, in hyponatremia, results in development of cerebral edema. If onset of hyponatremia is slow, the brain can adjust cell volume by decreasing intracellular osmolality and preventing influx of water from the ECF. Patients with chronic hyponatremia will also adjust intracellular osmolality to an extent that clinical signs may not be obvious even though sodium concentrations are quite low.

Treatment of hyponatremia varies with etiology of the disorder. The goals of therapy are to manage the underlying disease and, if necessary, to increase serum sodium and osmolality. Infusion with conventional crystalloid solutions (e.g., normal saline or lactated Ringer's solution) is reported to accomplish sodium and volume replacement in hyponatremic, hypovolemic patients (DiBartola, 1992c). Use of hypertonic saline solutions is not recommended since overly rapid correction of hyponatremia may do more harm than good. Chronic hyponatremia, in which the brain has adjusted to the decrease in osmolality and sodium, must be handled cautiously to avoid brain dehydration and injury, including osmotic demyelination syndrome. This syndrome, often occurring several days after correction of hyponatremia, results from areas of demyelination caused by treatment-induced increases in serum sodium concentration. Dogs with asymptomatic chronic hyponatremia are best treated by mild water restriction and monitoring of serum sodium. Chronic, symptomatic dogs should be treated such that the rate of increase of serum sodium does not exceed 10–12 mEq/l/day (0.5 mEq/l/h) (DiBartola, 1998). Again, the most important therapeutic goal in management of hyponatremia should be treatment of the underlying disease.

Hyperchloremia

Fluid loss associated with small bowel diarrhea often results in greater loss of HCO_3^- than chloride due to loss of alkaline pancreatic secretions and bile and HCO_3^- secretion in exchange for Cl^- in the ileum. The resulting hyperchloremic metabolic acidosis is characterized by a normal anion gap. Additional causes and treatment for hyperchloremic metabolic acidosis will be considered subsequently under the heading of metabolic acidosis. Please refer to the discussion of hypernatremia for treatment of hyperchloremia associated with loss of free water.

Hypochloremia

Hypochloremia may be seen in patients with fluid losses due to vomiting or excessive diuretic administration. Hypochloremic metabolic alkalosis may develop in these cases because an excess of chloride is lost, leading to decreased filtered Cl^- in the renal tubules. As previously noted, activity of the Na^+-K^+-$2Cl^-$ cotransporter in the luminal membrane of the macula densa cell is primarily determined by the availability of Cl^-. In hypochloremia, less Cl^- is delivered, resulting in less NaCl reabsorption, promotion of renin release leading to secondary hyperaldosteronism, and increased distal H^+ secretion. If further Na^+ reabsorption does occur, then Na^+ must be accompanied by an anion other than chloride, usually bicarbonate, or must be exchanged for a secreted cation, either H^+ or K^+. In addition, bicarbonate secretion in exchange for chloride, which is thought to occur in intercalated cells of the cortical collecting tubule, will decrease since this process is presumably driven by a favorable inward gradient for Cl^-. As luminal $[Cl^-]$ decreases, the gradient is dissipated and bicarbonate is retained in the system. All of the foregoing mechanisms promote retention of base and excretion of H^+, leading to a hypochloremic metabolic alkalosis (Rose, 1994). Treatment with chloride-replete fluid such as normal saline is usually adequate to resolve chloride-responsive alkalosis. Potassium depletion may also promote a metabolic alkalosis and should be addressed as needed by addition of potassium chloride to fluids.

Potassium

Homeostasis

As the major intracellular cation, potassium concentrations inside (145 mEq/l) and outside (3.5–5.5 mEq/l) the cell are maintained by the Na^+,K^+-ATPase pump. Under normal circumstances each pump actively transports three sodium ions out of and two potassium ions into the cell, but the ratio can change depending upon the circumstances. The ratio of intra- to extracellular concentration of potassium ($[K^+]_i/[K^+]_o$) is the major determinant of resting membrane potential. Resting membrane potential is crucial to normal membrane excitability associated with cardiac conduction, muscle contraction, and nerve impulse transmission.

The normal dietary intake of potassium is much more than the body requires. About 90% of this intake is excreted in the urine, with the remainder of what is not required eliminated in the stool. Plasma potassium concentration is determined by the movement of potassium into or out of cells. Two important factors stimulating the transport of potassium into cells are insulin and β-adrenergic stimulation (Clausen and Flatman, 1987).

Aldosterone is the primary determinant of potassium secretion across renal tubular epithelial surfaces.

Renal Regulation of Potassium Excretion

Most filtered potassium (60–80%) is reabsorbed in the proximal tubule. In the early proximal tubule, potassium enters the tubular cell at the luminal surface by active transport. The intracellular concentration of potassium is high, and the lumen of the tubule is negatively charged relative to the interior of the early proximal tubular cell. Potassium passively exits the basolateral membrane of the tubular cell down a favorable chemical concentration gradient. In the mid-to-late proximal tubule, the tubular lumen is relatively more positively charged than the tubular cell interior. This favors the passive reabsorption of potassium. Potassium again exits on the basolateral side of the tubular cell down a concentration gradient. Potassium reabsorption by intercalated cells in the distal nephron is similar to the process in the early proximal tubule and involves active transport at the luminal cell membrane followed by passive diffusion from the cell at the basolateral membrane. Tubular secretion of potassium is aldosterone mediated and occurs in the distal nephron (late distal tubule or connecting tubule of the collecting duct system) primarily in the "principal" cells of the collecting tubules. Additional information on mechanisms of collecting duct system reabsorption and secretion is given in Chapter 24 (Figure 24.2). Principal cells are rich in Na^+,K^+-ATPase and respond to aldosterone by increasing the number and activity of Na^+,K^+-ATPase pumps in the basolateral membrane. The increasing luminal membrane permeability to sodium causes greater lumen negativity relative to the tubular cell interior and increases luminal permeability to potassium. This facilitates potassium secretion into the tubule lumen. Aldosterone-stimulated Na^+,K^+-ATPase actively pumps potassium out of the peritubular fluid through the basolateral tubular cell membrane. Movement of potassium from the tubular cell through the luminal membrane and into the tubule lumen is favored by relative negativity of the lumen compared to the interior of the distal tubule cell (Black, 1993).

When plasma potassium concentration is low, secretion of potassium by the principal cells is reduced while hydrogen ion secretion may be increased. Active potassium reabsorption by intercalated cells in the distal nephron is also stimulated by a potassium deficit. An additional factor affecting the movement of potassium across tubular cells is related to tubular flow rate. A rapid flow of filtrate through the tubules maximizes the potassium concentration gradient between the tubular cell interior and the lumen of the tubule and enhances potassium excretion. A reduction of tubular flow slows secretion by allowing a relatively greater concentration of potassium to be maintained in the lumen of the distal tubule (DiBartola and Autran de Morais, 1992).

Disorders of Potassium Balance

Disorders of potassium balance have marked effects on excitable membranes. The difference between the resting membrane potential and the membrane potential required for depolarization (threshold potential) determines the excitability of a cell. Hypokalemia makes the resting membrane potential more negative, thereby hyperpolarizing the cell and increasing the difference between resting and threshold potentials. Hyperkalemia causes the resting membrane potential to become more positive, hypopolarizing the cell and causing hyperexcitability. In hyperkalemia, if the resting potential decreases to less than the threshold potential, the cell depolarizes but is incapable of repolarizing, resulting in loss of cell excitability (DiBartola and Autran de Morais, 1992). In cardiac muscle this results in diastolic arrest; in vascular smooth muscle hyperkalemia causes vasoconstriction.

Changes in pH affect the distribution of potassium between the ICF and the ECF. When acidosis is present, potassium moves out of cells in exchange for hydrogen, which moves intracellularly. In the distal tubule more hydrogen, and relatively less potassium, may be exchanged for sodium at the luminal membrane, leading to decreased potassium excretion. Based on these general principles, a clinical rule of thumb predicts that each 0.1 unit decrease in pH will be accompanied by a 0.6 mEq/l increase in serum potassium concentration.

Conversely, in alkalosis potassium tends to move into cells in exchange for extracellular movement of hydrogen. Hypokalemia has been thought to promote alkalosis because less potassium is available to be exchanged for sodium in the distal tubule. Instead, sodium exchanges for hydrogen at the luminal membrane, leading ultimately to reclamation of bicarbonate and increased systemic pH. At the same time that systemic pH is increasing, secreted hydrogen ions exchanged for sodium cause the urine pH to decline.

Although the principles outlined above are commonly stated and widely applied clinically, it is not clear that these explanations are adequate. In acidosis, the effect of pH changes on potassium translocation varies with the nature of the acid anion, blood pH and HCO_3^- concentration, osmolality, hormonal activity, and liver and renal function (DiBartola and Autran de Morais, 1992). Although changes in serum potassium have been documented during acute mineral acidosis caused by HCl or NH_4Cl (Adrogue and Madias, 1981), acute metabolic acidosis caused by organic acids did not increase serum potassium as predicted (Oster et al., 1980; Adrogue and Madias, 1981). In certain conditions (e.g., diabetic ketoacidosis), hyperkalemia may be more directly associated with hyperosmolality and insulin deficiency than with the acidosis itself. In lactic acidosis, increased serum potassium concentration may be the result of release of intracellular potassium caused by cell breakdown associated with decreased peripheral perfusion (Black, 1993). Metabolic acidosis associated with both mineral and organic acids may directly or indirectly stimulate aldosterone secretion. The effects of aldosterone facilitate excretion of the acid load and, presumably, potassium, although one study failed to show any changes in serum potassium concentration (Perez et al., 1980).

Early studies of the effects of hypokalemia on acid–base balance may have overlooked the key role of chloride depletion in causing metabolic alkalosis (DiBartola and Autran de Morais, 1992). When pure potassium depletion is created iatrogenically in rats, metabolic alkalosis results. However, in dogs, potassium deficit with normal chloride levels leads to metabolic acidosis due, presumably, to a distal renal tubular acidification defect (Garella et al., 1979).

Hyperkalemia

Total body potassium may be normal, decreased, or increased with hyperkalemia. Clinical signs of hyperkalemia (>7.5 mEq/l) are generally associated with changes in membrane excitability and are more severe if the increase in potassium has been rapid. Muscle weakness, twitching, and irritability may occur. Electrocardiographically determined cardiac effects may include extrasystoles, intraventricular conduction blocks, high-peaked T waves, altered QT interval, widened QRS interval, decreased amplitude or disappearance of P waves, depressed ST segment, ventricular asystole, or fibrillation.

Causes of hyperkalemia are summarized in Table 23.6. The more common causes are related to decreased urinary potassium excretion. Pseudohyperkalemia related to hemolysis can occur in species that have high red cell potassium concentrations similar to humans. Dogs, sheep, and cattle can be divided into two groups based on Na$^+$,K$^+$-ATPase activity in red cell membranes. Those animals with high activity and high intracellular potassium concentrations are at risk for hyperkalemia caused by hemolysis. Animals with genetically determined low activity and low intracellular concentrations of potassium are unlikely to suffer from pseudohyperkalemia since the concentration of potassium in red cells resembles the concentration in the ECF (DiBartola and Autran de Morais, 1992).

The effects of several different drugs may impact serum potassium concentration. Since potassium uptake by cells is mediated in part by catecholamines at β receptors, beta blockers decrease intracellular potassium movement and increase ECF potassium concentrations. Angiotensin-converting enzyme (ACE) inhibitors may cause hyperkalemia by interfering with angiotensin II–mediated aldosterone secretion. Prostaglandin inhibitors, heparin, and selected potassium-sparing diuretics (e.g., spironolactone) increase serum potassium by decreasing the secretion of aldosterone or by blocking its activity. In many cases drugs alone may not have a marked effect on serum potassium concentration but if combined with a potassium load or decreased renal function may cause clinically significant hyperkalemia.

Table 23.6 Causes of hyperkalemia. Source: Adapted from DiBartola, 1992a. Reproduced with permission of Elsevier.

Decreased excretion
- Urethral obstruction
- Ruptured bladder
- Anuric or oliguric renal failure
- Hypoadrenocorticism
- Gastrointestinal diseases (e.g., trichuriasis, salmonellosis, perforated duodenal ulcers)
- Chylothorax with repeated drainage of the pleural effusion
- Drugs
  - ACE inhibitors (e.g., captopril, enalapril)
  - Potassium-containing drugs (e.g., potassium chloride)
  - Potassium-sparing diuretics (e.g., spironolactone, amiloride, triamterene)
  - Nonsteroidal antiinflammatory agents
  - Heparin

Translocation from the intracellular to extracellular fluid
- Acute mineral acidosis (e.g., HCl or NH_4Cl administration)
- Insulin deficiency (e.g., diabetic ketoacidosis)
- Ischemia reperfusion
- Drugs (e.g., propranolol)
- Acute tumor lysis syndrome
- Hyperkalemic periodic paralysis (rare)

Increased intake (rare)

Pseudohyperkalemia
- Thrombocytosis
- Hemolysis

Treatment of hyperkalemia varies with the severity of the condition in terms of magnitude and rapidity of onset. Emergency treatment is indicated if potassium rises quickly and exceeds 6.0–8.0 mEq/l (Phillips and Polzin, 1998). Serum potassium concentrations less than these do not typically induce life-threatening cardiotoxicity and can usually be managed with administration of potassium-free fluids. More aggressive treatment is necessary if electrocardiographic signs suggest toxicity. Additional measures that may be taken in treatment of severe hyperkalemia are summarized in Table 23.7. Some are directed toward increasing movement of potassium from the extracellular to the intracellular compartment (i.e., glucose, insulin, and sodium bicarbonate), while others are intended to decrease potassium from the ECF by enhanced renal excretion (e.g., diuretics) or decreased gastrointestinal absorption (i.e., orally

Table 23.7 Therapeutic considerations in the management of severe hyperkalemia

- Establish venous access and administer potassium-deficient fluids
- Discontinue potassium intake, including drugs that may promote hyperkalemia
- Administer the following as needed:
  - $NaHCO_3$ (0.5–1 mEq/kg, slowly IV) if animal is acidotic
  - Calcium gluconate (10% solution; 0.5–1 ml/kg slowly IV up to 10 ml maximum)
  - Glucose (20% solution; 0.5–1.0 g/kg IV)
  - Insulin (0.5 IU/kg) and glucose (20% solution; l g/kg; half given IV bolus and the remainder infused over 2 hours)
- Potassium-wasting diuretics (furosemide, chlorothiazide, hydrochlorothiazide)
- Sodium polystyrene (20 g with 100 ml 20% sorbitol) per os or 50 g in 100–200 ml tap water (retention enema)
- Peritoneal dialysis (last resort)

administered potassium-binding resins such as sodium polystyrene sulfonate).

Therapy with calcium gluconate is included as part of the emergency treatment of hyperkalemia because changes in membrane excitability associated with alterations in potassium may be exacerbated by abnormalities in ionized calcium. Ionized calcium affects the threshold potential of a membrane and, when calcium is decreased, brings threshold closer to resting membrane potential, resulting in greater membrane excitability. An increase in ionized calcium has an opposing effect on membrane excitability by increasing the threshold potential and making depolarization more difficult. Hence, hypocalcemia exacerbates hyperkalemia while hypercalcemia counteracts hyperkalemia.

Hypokalemia

Since 97% of total body potassium is intracellular, depletion can occur with no change in plasma potassium concentration or even with an increase if acidosis is present. Clinical signs of hypokalemia (<2.5–3.0 mEq/l) can include weakness of skeletal and respiratory muscles and loss of intestinal smooth muscle tone. As in hyperkalemia, cardiac changes occur as potassium concentration changes. Supraventricular and ventricular arrhythmias are most commonly observed in animals. ECG hallmarks of hypokalemia in humans are flattened or inverted T waves, depressed S-T segment, and the appearance of U waves. Prolongation of the QT interval and U waves have been reported in dogs but are not as consistently seen as they are in humans. Hypokalemia is increasingly recognized as an important clinical problem in cats, especially in association with chronic renal failure and geriatric animals (Phillips and Polzin, 1998). Feline hypokalemic polymyopathy syndrome, characterized by generalized muscle weakness associated with hypokalemia, is often manifest in cats as ventroflexion of the head and a stiff, stilted gait.

Table 23.8 Causes of hypokalemia. Source: Adapted from DiBartola, 1992a. Reproduced with permission of Elsevier.

- *Increased loss*
  - Gastrointestinal (FEK <4–6%)
    - Persistent vomiting of stomach contents
    - Diarrhea
  - Urinary (FEK >4–6%)
    - Chronic renal failure in cats
    - Diet-induced hypokalemic nephropathy in cats
    - Renal tubular acidosis
    - Postobstructive diuresis
  - Excess circulating mineralocorticoid
    - Hyperadrenocorticism
    - Primary hyperaldosteronism (hyperplastic or neoplastic)
    - Diuretics (loop acting, thiazides and osmotic)
    - Antibiotics (penicillins, amphotericin B, aminoglycosides)
- *Translocation from extracellular fluid to intracellular*
  - Alkalemia
  - Overadministration of insulin and glucose-containing fluids
  - Hyperthyroidism
  - Hypokalemic periodic paralysis
  - Possible complication of hypothermia
- *Decreased intake*
  - Unlikely as sole cause

Increased loss associated with the gastrointestinal or the urinary system is a common cause of hypokalemia, as indicated in Table 23.8. Differentiating gastrointestinal from urinary causes of hypokalemia is largely accomplished by clinical signs and physical exam, but fractional potassium excretion rates (FE_K) may also be useful. Fractional potassium excretion can be calculated using the following formula:

$$FE_K = \left(U_K/S_K\right) / \left(U_{CR}/S_{CR}\right) \times 100$$

where U indicates the urine concentration of potassium (K^+) or creatinine (CR), and S indicates the serum concentration.

Treatment of hypokalemia is indicated if significant potassium loss is expected based on history and clinical signs (e.g., vomiting, diarrhea, overzealous use of diuretics) or if clinical signs of hypokalemia are present. Appropriate potassium administration is often required with prolonged fluid therapy. If feasible, oral potassium supplementation is most desirable since this is the safest route of administration. If intravenous potassium supplementation is warranted, the amount administered should be based on clinical status of the animal and measured serum potassium values. Table 23.9 provides approximate potassium dosages for treatment of hypokalemia in small animals. Alternatively, a rule of thumb may be applied in which 20 mEq/l of potassium is supplemented with careful monitoring of changes in serum potassium. An important caution regarding the administration of intravenous potassium is not to exceed a rate

Table 23.9 Potassium supplementation in treatment of hypokalemia

Serum potassium concentration (mEq/l)	Supplement fluids (mEq/l)[a]
3.5 to 4.5	20
3.0 to 3.5	30
2.5 to 3.0	40
2.0 to 2.5	60
<2.0	80

[a] Quantity of potassium to add per liter of fluid. Do not exceed administration rate of 0.5 mEq K^+/kg/h.

of 0.5 mEq/kg/h. Parenteral potassium administration should always be monitored to ensure that rate of potassium addition does not exceed rate of potassium movement into cells.

Principles of Acid–Base Metabolism

Homeostasis

Blood pH is highly regulated and is normally maintained between 7.38 and 7.42. Pulmonary and renal functions are necessary for precise regulation of pH of all body fluids, blood, and extravascular tissues. An acid is defined by Bronsted and Lowry as a substance that can supply H^+ (protons), and a base is defined as a substance that can accept H^+. In aqueous solutions, H^+ are hydrated; therefore, H_3O^+ is considered an acid and is implied by the symbol H^+. Blood pH is the negative logarithm of the hydrogen ion concentration. Although hydrogen ion concentration cannot be measured directly, hydrogen ion activity is measured chemically using a pH electrode. In body fluids, the difference between activity of hydrogen ions and concentration of hydrogen ions is negligible; hence hydrogen ion concentration and pH are commonly referred to in acid–base discussions. The hydrogen ion concentration of blood at pH 7.4 is 40 nmol/l (nanoequivalents per liter) and is therefore approximately a millionfold lower than the blood concentration of electrolytes such as sodium and potassium. Appropriate hydrogen ion concentration is critical in order to maintain body proteins in configurations required for enzymatic and structural function. An increase in hydrogen ion concentration with a decrease in blood pH is termed acidemia and can be caused by pathophysiological processes that cause accumulation of acids in the body. As the concentration of hydrogen ions decreases, and blood pH increases, alkalemia occurs and can be associated with pathophysiological processes that cause accumulation of alkali in the body. The disordered processes leading to acidemia and alkalemia are termed acidosis and alkalosis, respectively.

On a daily basis, an excess of acid (70–100 mEq) is generated in the body as a result of dietary intake and intermediary metabolism. Catabolism of carbohydrate, fat, and protein account for most of this as a result of: oxidation of sulfur-containing amino acids to sulfuric acid; oxidation of phosphoproteins to phosphoric acid; incomplete oxidation of fats and carbohydrates to organic acid; production of lactate/lactic acid during anaerobic glycolysis; and conversion of carbon dioxide and water produced in the tricarboxylic acid cycle to carbonic acid. Buffers throughout the body minimize changes in blood pH associated with alterations of acid–base balance. The most effective physiological buffers have pK_a values between 6.1 and 8.4, with buffering capacity being maximal within one pH unit of the pK_a. Important extracellular buffers include bicarbonate, inorganic phosphates, and plasma proteins.

Most extracellular buffering occurs as a result of the bicarbonate–carbonic acid buffer pair (pKa = 6.1). Equilibrium of this buffer pair is indicated with the following:

$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

The hydration of CO_2 is a rapid reaction in the presence of the enzyme carbonic anhydrase, which is found primarily in red blood cells and renal tubular cells. The dissociation of any acid, in this case carbonic acid, can be described utilizing the concept that the velocity of a reaction is proportional to the product of the concentration of the reactants. In the case of the bicarbonate buffer system, the carbonic anhydrase–catalyzed hydration of CO_2 to form H_2CO_3 reaches equilibrium almost instantaneously, with the number of dissolved CO_2 molecules far exceeding the number of carbonic acid molecules. By defining dissociation constants and rearranging, the useful Henderson–Hasselbalch form of the dissociation equilibrium equation can be derived:

$$\mathrm{pH} = \mathrm{pKa} + \log\left[HCO_3^-\right] / \left[H_2CO_3\right]$$

Gaseous CO_2 produced in the tissues, primarily via the tricarboxylic acid cycle, is soluble in water; the concentration of dissolved CO_2 in body fluids can be related to the partial pressure of CO_2 in the gas phase, $P\mathrm{CO}_2$, by the following expression:

$$\left[\text{dissolved } CO_2\right] = 0.03 \times P_{CO_2}$$

Hence the clinically useful form of this equation for the bicarbonate–carbonic acid buffer system becomes

$$\mathrm{pH} = 6.1 + \log\left[HCO_3^-\right] / (0.03 \times P_{CO_2})$$

The bicarbonate–carbonic acid system is the most physiologically important extracellular buffer system because it is present in relatively high concentrations in the blood and because it can effectively buffer by rapid regulation of $P\mathrm{CO}_2$ through alveolar ventilation. As carbonic acid is formed from the buffering of excess H^+ by HCO_3^-, this drives the dissociation equation of carbonic acid to the left, causing an increase in $P\text{CO}_2$. An increase in ventilation enhances CO_2 excretion and lowers the $P\text{CO}_2$.

Intracellular buffers also contribute to maintenance of body pH. The primary intracellular buffers are proteins, organic and inorganic phosphates, and, in the red cell, hemoglobin. Hemoglobin is an especially important buffer for carbonic acid since the primary extracellular buffer, the bicarbonate system, cannot buffer this acid. Bone also acts as a tissue-based buffer by exchanging surface Na^+ and K^+ for H^+ under conditions of acid load. Additionally, dissolution of bone mineral results in release of buffer compounds into the ECF.

Regulation of Hydrogen Ion, Carbon Dioxide, and Bicarbonate

Pulmonary and renal control of dissolved CO_2 and bicarbonate concentrations, respectively, is responsible for maintenance of body pH. The "tail" of the Henderson–Hasselbalch equation for the bicarbonate system (i.e., HCO_3^-/dissolved CO_2) provides a simplistic but useful means to consider pulmonary and renal adjustments during simple acid–base disturbances. Under normal physiological conditions, the ratio of HCO_3^- to dissolved CO_2 is 20 : 1. This ratio can be disturbed by addition or loss of CO_2 or bicarbonate to the system. Table 23.10 depicts changes in the tail of the bicarbonate–carbonic acid dissociation equation that might occur during simple acid–base disturbances. The respiratory component of acid–base regulation (the denominator of the tail, or dissolved CO_2) involves changes in respiratory rate and volume prompted by changes in $P\text{CO}_2$. Initiation of these processes requires only minutes.

The renal component of acid–base regulation (the numerator of the tail, or HCO_3^-) involves selective absorption of bicarbonate and secretion of H^+. During periods of acidosis, relatively more H^+ are secreted, while relatively more K^+, Na^+, and HCO_3^- are retained. During alkalosis, K^+ is secreted, while relatively more H^+ and less Na^+ and HCO_3^- are retained. This process requires hours to days to produce an effect. The kidney regulates acid–base balance by maintaining the appropriate HCO_3^- in the plasma. The kidney accomplishes this by reclaiming virtually all filtered HCO_3^- and excreting an amount of acid that equals the amount of ingested or endogenously generated nonvolatile acid. In the proximal tubule of the kidney, cytoplasmic carbonic anhydrase catalyzes the formation of H^+ and bicarbonate from cellular carbon dioxide and water, controlling the rate of hydrogen secretion and bicarbonate reabsorption. In the luminal membrane, carbonic anhydrase converts carbonic acid to carbon dioxide and water, increasing net bicarbonate reabsorption (Figure 23.5A). In the distal nephron, intercalated cells specialized for hydrogen secretion contain large quantities of carbonic anhydrase, again yielding hydrogen and bicarbonate. In this case, secreted H^+ serves to titrate buffers in the urine (phosphate buffering is shown in Figure 23.5B) and lower urinary pH. As titratable acidity of the urine reaches a maximum, another adaptation, increased ammonia (NH_3) production by tubular cells, contributes to excretion of acid loads. Figure 23.5C, shows production of freely

Table 23.10 Examples of changes in the "tail" of the Henderson–Hasselbalch equation occurring during simple acid–base disturbances

Respiratory acidosis (↓ CO_2 elimination) associated with inadequate ventilation:

$$\underset{\text{(Normal)}}{\frac{20\text{HCO}_3^-}{1\text{CO}_2}} + \underset{(\downarrow\ \text{Ventilation})}{2\text{CO}_2} \rightarrow \underset{\text{(Uncompensated)}}{\frac{20\text{HCO}_3^-}{3\text{CO}_2}} + \underset{\text{(Renal production)}}{40\text{HCO}_3^-} \rightarrow \underset{\text{(Compensated)}}{\frac{60\text{HCO}_3^-}{3\text{CO}_2}} = \frac{20}{1}$$

Respiratory alkalosis (↑ CO_2 elimination) associated with hyperventilation:

$$\underset{\text{(Normal)}}{\frac{20\text{HCO}_3^-}{1\text{CO}_2}} - \underset{(\uparrow\ \text{Ventilation})}{0.5\text{CO}_2} \rightarrow \underset{\text{(Uncompensated)}}{\frac{20\text{HCO}_3^-}{0.5\text{CO}_2}} - \underset{\text{(Renal excretion)}}{10\text{HCO}_3^-} \rightarrow \underset{\text{(Compensated)}}{\frac{10\text{HCO}_3^-}{0.5\text{CO}_2}} = \frac{20}{1}$$

Metabolic acidosis (bicarbonate deficit) associated with diarrhea:

$$\underset{\text{(Normal)}}{\frac{20\text{HCO}_3^-}{1\text{CO}_2}} - \underset{\text{(Loss in feces)}}{10\text{HCO}_3^-} \rightarrow \underset{\text{(Uncompensated)}}{\frac{10\text{HCO}_3^-}{2\text{CO}_2}} - \underset{(\text{Eliminated by}\ \uparrow\ \text{ventilation})}{0.5\text{CO}_2} \rightarrow \underset{\text{(Compensated)}}{\frac{10\text{HCO}_3^-}{0.5\text{CO}_2}} = \frac{20}{1}$$

Metabolic alkalosis (bicarbonate excess) associated with administration of alkali:

$$\underset{\text{(Normal)}}{\frac{20\text{HCO}_3^-}{1\text{CO}_2}} + \underset{\text{(Alkali administration)}}{20\text{HCO}_3^-} \rightarrow \underset{\text{(Uncompensated)}}{\frac{40\text{HCO}_3^-}{1\text{CO}_2}} - \underset{(\text{Eliminated by}\ \downarrow\ \text{ventilation})}{1\text{CO}_2} \rightarrow \underset{\text{(Compensated)}}{\frac{40\text{HCO}_3^-}{2\text{CO}_2}} = \frac{20}{1}$$

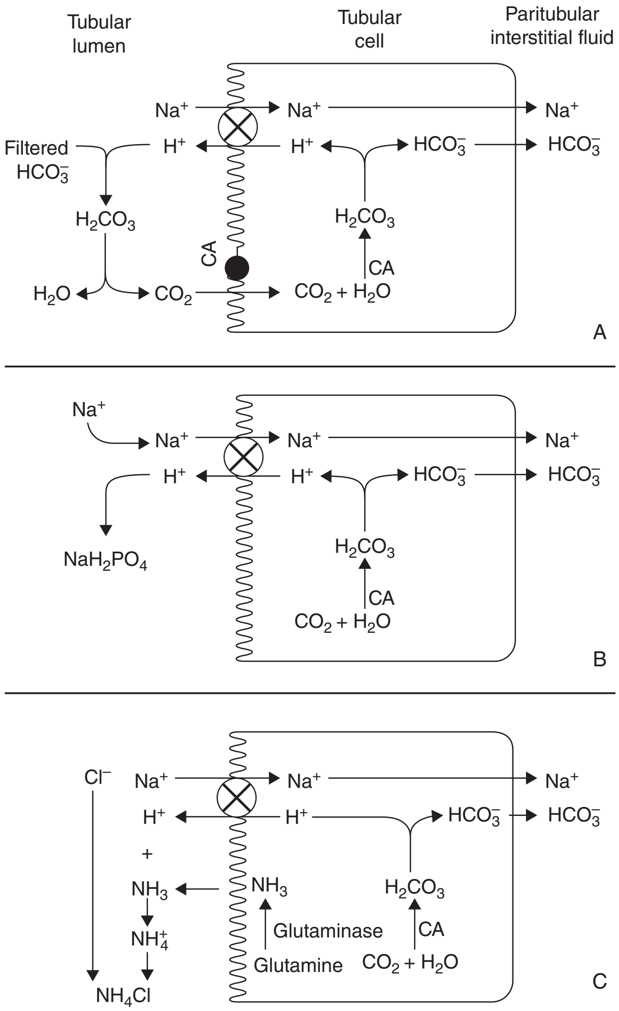


Figure 23.5 Renal mechanisms for H^+ excretion. See text for explanation of each panel.

diffusable NH_3 from glutamine moving into the tubular lumen, where it combines with H^+ to form ammonium (NH_4^+). Ammonium, in turn, combines with chloride for excretion as ammonium chloride. While this is an oversimplification of the physiological events, it is acceptable to consider ammonium chloride as a flexible mechanism for H^+ secretion based on the ability of the kidney to generate ammonia.

Assessment of Acid–Base Disturbances

Disorders of acid–base equilibrium can result from a primary disturbance in pulmonary regulation of the concentration of H_2CO_3 in body fluids via changes in alveolar ventilation and $P\text{CO}_2$ levels, from metabolic changes in concentration of bicarbonate, or from a combination of these mechanisms.

The partial pressure of CO_2 ($P\text{CO}_2$) is generally accepted as the best measure of respiratory disturbances. Assessment of $P\text{CO}_2$ depends upon availability of a blood gas analyzer and proper arterial sample collection. A blood gas analysis provides three measured parameters (pH, $P\text{CO}_2$, $P\text{O}_2$) and typically two calculated values (actual bicarbonate and base excess). Acidemia and alkalemia (using pH), eucapnia, hypercapnia or hypocapnia (using $P\text{CO}_2$), and hypoxemia (using $P\text{O}_2$ if the sample is arterial) may be directly assessed. In-house blood gas and electrolyte analyzers have become much more common in practice, making assessment of these parameters practical and economical. Results obtained with one handheld analyzer appropriate for in-house testing were similar to those obtained from a standard chemistry analyzer with the exception of sodium concentration in canine samples and hematocrit in equine samples (Looney et al., 1998).

Actual bicarbonate values are useful in assessment of nonrespiratory disorders, but these values will vary with compensatory changes in alveolar ventilation and $P\text{CO}_2$. Bicarbonate values are derived using the Henderson–Hasselbalch equation and measured values for pH and $P\text{CO}_2$. Plasma bicarbonate values may also be estimated by measurement of total CO_2. Total CO_2 combines measurement of both the numerator and the denominator of the tail of the Henderson–Hasselbalch equation ($[HCO_3^-]/[H_2CO_3]$) by converting both to measurable CO_2. Total CO_2 and plasma bicarbonate are used interchangeably in reporting plasma bicarbonate concentrations even though total CO_2 is actually plasma bicarbonate plus 1.1–1.3 mEq of H_2CO_3. As compared to actual plasma bicarbonate, standard bicarbonate is defined as the concentration of bicarbonate after fully oxygenated whole blood has been equilibrated with CO_2 at a $P\text{CO}_2$ of 40 mmHg at 38°C; this measurement eliminates the influence of respiration on plasma HCO_3^-.

Standard base excess (BE) is the concentration of titratable base of ECF; this value may be calculated using a Siggaard–Anderson alignment nomogram that interrelates BE and total CO_2 and HCO_3^- when pH and $P\text{CO}_2$ are measured. Because this calculation is based on a constant oxygen saturation, error may be introduced by inclusion of air bubbles in a poorly handled blood sample. In veterinary medicine, error may also be inherent because the nomogram is based on human blood and excludes the effects of plasma protein and electrolytes on acid–base equilibrium. BE is useful because it accounts for the effects of CO_2 on carbonic acid equilibrium and identifies nonrespiratory causes of acid–base derangement. Base deficit is defined as the negative of base excess (Bailey and Pablo, 1998).

Anion Gap

Further analysis, beyond pH, $P\text{CO}_2$, HCO_3^-, and BE, may be useful in assessment of complex acid–base disturbances. The anion gap (AG) is defined as the difference between the quantity of unmeasured cations (UCs) and unmeasured anions (UAs) in the blood. Major UAs include phosphates, sulfates, and organic acids (e.g., lactate, citrate, ketones), with chloride and bicarbonate being the measured anions. Major UCs include calcium and magnesium, with sodium and potassium being the measured cations. Calculation of the AG according to the following equations reflects the law of electroneutrality, according to which total cations must equal total anions (DiBartola, 1992d).

$$[\mathrm{Na}^+] + [\mathrm{K}^+] + [\mathrm{UC}] = [\mathrm{Cl}^-] + [\mathrm{HCO}_3^-] + [\mathrm{UA}]$$

$$\text{Anion gap} = \mathrm{UC} - \mathrm{UA} = ([\mathrm{Na}^+] + [\mathrm{K}^+]) - ([\mathrm{Cl}^-] + [\mathrm{HCO}_3^-])$$

The normal AG varies with the species but is approximately 13–24 mEq/l in dogs and cats. AG is most often used to identify causes of metabolic acidosis. In organic acidoses, $\mathrm{HCO_3}^-$ buffers hydrogen ions that are generated from dissociation of organic acid (e.g., lactic acid). In theory, the measured $[\mathrm{HCO_3}^-]$ should decrease as the concentration of the UA (the organic acid) increases. As long as $[\mathrm{Cl}^-]$ remains unchanged (normochloremic metabolic acidosis), the gap will increase proportionately with the increase in acid. Several factors that may confound this simple relationship include the following: (i) other buffers besides $\mathrm{HCO_3}^-$ also respond to the influx of organic acid; (ii) the volume of distribution of $\mathrm{HCO_3}^-$ may be different from that of the acid; and (iii) the patient's AG baseline (prior to the presenting illness) is often not known. Hence the AG is useful but not fully predictable.

Increased AG often occurs in lactic acidosis, diabetic ketoacidosis, azotemic renal failure (due to increased phosphates and sulfates), and poisoning (ethylene glycol, salicylate). Constable et al. (1997) demonstrated a useful correlation between AG and serum creatinine concentration in calves with experimentally induced diarrhea and adult cattle with abomasal volvulus. Although the AG was not a useful predictor of all anion-associated changes (e.g., no correlation was found between AG and blood lactate levels), the AG could alert clinicians to the potential presence of uremic acidosis.

A normal AG usually occurs in metabolic acidosis related to diarrhea, renal tubular acidosis, excessive use of carbonic anhydrase inhibitors, or ammonium chloride administration and in iatrogenic expansion acidosis caused by excessive normal saline administration. The two most common causes of a decreased AG are hypoalbuminemia or dilution of plasma proteins caused by infusion of crystalloid solutions. In both cases the gap decreases as a result of a decreased concentration of net negative charges associated with plasma proteins. Each 1.0 g/dl decrease in albumin is associated with an approximately 2.4 mEq/l decrease in the AG (Gabow, 1985).

Nontraditional (Stewart's) Acid–Base Analysis

An understanding of the traditional interrelationships between H^+, $\mathrm{CO_2}$, and $\mathrm{HCO_3}^-$ is adequate to explain the behavior of aqueous solutions; however, it does not account for the effects of plasma proteins and electrolytes, particularly sodium and chloride, on acid–base status in biological systems. Stewart described a new approach to understanding acid–base physiology based on three fundamental concepts of electrolyte chemistry (Stewart, 1978, 1983). First, electroneutrality must always be maintained. Hence, as with the concept of the AG, the sum of all positive charges must equal the sum of all negative charges. Second, mass must be conserved even though it may change in form within a solution. Finally, the dissociation or ionization of a substance in water is determined by its dissociation constant. Weak electrolytes relevant to acid–base physiology include proteins, water, and $\mathrm{CO_2}$. In contrast, sodium and chloride are considered strong electrolytes because they are fully dissociated in water. Evaluation of acid–base status using the Stewart approach requires assessment of independent, or primary, variables; dependent, or unknown, variables; and dissociation constants of all variables. Values of independent variables are controlled externally and cannot be changed by processes occurring within the solution. Independent variables dictate the acid–base status of a solution.

The independent variables controlling acid–base status in biological solutions are strong ion difference (SID), $P\mathrm{CO_2}$, and total weak acid concentration ($\mathrm{A_{TOT}}$). The first variable, SID, is the sum of the strong cation concentrations minus the sum of the strong anion concentrations:

$$\mathrm{SID} = ([\mathrm{Na}^+] + [\mathrm{K}^+]) - ([\mathrm{Cl}^-] + [\text{lactate}^-] + [\text{ketoacid}])$$

Unless lactic acidosis or ketoacidosis is suspected in a given case, these terms may be eliminated from the equation since their values would be quite small. Likewise, $[\mathrm{K}^+]$ is often dropped from the equation since it contributes a relatively small number to the total cation population. If $P\mathrm{CO_2}$ and $\mathrm{A_{TOT}}$ remain constant, increases in SID suggest nonrespiratory alkalosis and decreases suggest nonrespiratory acidosis. Mean normal SID values are derived by each laboratory based on their reference population, and these values vary across species. The second independent variable, $P\mathrm{CO_2}$, is an indication of the amount of $\mathrm{CO_2}$ dissolved in plasma. As in traditional acid–base theory, an increase in $P\mathrm{CO_2}$ shifts the dissociation equation for carbonic acid to the right, increasing the $[\mathrm{H}^+]$ and making the solution more acidic. The final independent variable, $[\mathrm{A_{TOT}}]$, is accounted for by plasma proteins (95%), primarily albumin, and inorganic phosphates (5%). $\mathrm{A_{TOT}}$ has been calculated for horses (Constable, 1997) using the formula

$$[\mathrm{A_{TOT}}]\ (\mathrm{mEq/l}) = 2.25\ [\mathrm{albumin}]\ (\mathrm{g/dl}) + 1.4\ [\mathrm{globulin}]\ (\mathrm{g/dl}) + 0.59\ [\mathrm{phosphate}]\ (\mathrm{mg/dl})$$

These three independent variables influence several dependent, or unknown, variables. Dependent variables are affected by processes occurring within the solution and do not change unless independent variables change. Values for dependent variables are thus the result, not the cause, of events in solution. Dependent variables include $[\mathrm{H^+}]$, $[\mathrm{HCO_3^-}]$, carbonate ion concentration ($[\mathrm{CO_3^{2-}}]$), $[\mathrm{OH^-}]$, concentration of dissociated weak acids ($[\mathrm{A^-}]$), and concentration of nondissociated weak acids ([AH]). Values of dependent variables are not affected by the values of other dependent variables. Because the values for $[\mathrm{CO_3^{2-}}]$ and $[\mathrm{OH^-}]$ are so small, they are not measured or evaluated in a clinical setting. The variables for dissociated and nondissociated weak acids reflect the dynamic relationship between acid–base balance and protein ionization. The ability of proteins to function as enzymes, cell membrane pumps, ion channels, receptors, etc., depends upon their state of ionization, and this is directly affected by changes in independent variables ($P\mathrm{CO_2}$, SID, and $\mathrm{A_{TOT}}$). Likewise, the ratio of ionized to unionized calcium depends upon protein binding, which changes with alterations of $\mathrm{A_{TOT}}$ and pH.

Independent variables are controlled via respiration ($P\mathrm{CO_2}$) and renal function (SID). As in traditional acid–base theory, rate and depth of respiration control retention or elimination of $\mathrm{CO_2}$, which may lead to respiratory acidosis or alkalosis, respectively. Control of SID is primarily accomplished by the kidney with a smaller contribution from the gastrointestinal tract. Changes in SID via the kidneys are achieved much more slowly than respiratory changes and are on the order of hours to days. The kidney regulates SID by differential reabsorption of $\mathrm{Na^+}$ and $\mathrm{Cl^-}$. Since $\mathrm{Na^+}$ reabsorption is strongly related to renal regulation of ECF volume, net $\mathrm{Cl^-}$ excretion relative to net $\mathrm{Na^+}$ excretion is the primary mechanism for renal regulation of acid–base balance. Control of $P\mathrm{CO_2}$ and SID is the primary determinant of acid–base balance because there is no evidence that the body alters the third independent variable, protein concentration $[\mathrm{A_{TOT}}]$, in order to regulate acid–base balance.

In summary, the most important premise of Stewart's approach is that concentrations of $\mathrm{HCO_3^-}$ and $\mathrm{H^+}$ are dependent on concentrations of primary, or independent, variables, notably $\mathrm{CO_2}$, $\mathrm{Na^+}$, and $\mathrm{Cl^-}$. The complex equations derived by Stewart address the changes induced by independent variables and quantitate each potential influence by solving for the dependent variables. Much simplified versions of Stewart's formula have been adopted on a limited basis by clinicians who value Stewart's theories and believe that they provide a more complete picture of acid–base derangements. Table 23.11 summarizes the equations being applied for nontraditional analysis of nonrespiratory acid–base status (Russell et al., 1996). In brief, increases in SID suggest nonrespiratory alkalosis, whereas decreases suggest nonrespiratory acidosis. Negative values for Δ albumin suggest hyperproteinemic acidosis, whereas positive values reflect hypoproteinemic alkalosis. Negative values for Δ phosphorus suggest hyperphosphatemic acidosis. Negative changes in free water point to dilutional acidosis, and positive values suggest concentration alkalosis. Positive values for Δ chloride suggest hypochloremic alkalosis, and negative values suggest hyperchloremic acidosis.

Table 23.11 Formulas for quantitative analysis of nonrespiratory acid–base status

I. Estimation of [SID] (all values expressed as mEq/l)
   $[\mathrm{SID\ approx.}] = [\mathrm{Na^+}_{\mathrm{mean\ normal}}] - [\mathrm{Cl^-}_{\mathrm{corrected}}]$
   $[\mathrm{Cl^-}_{\mathrm{corrected}}] = [\mathrm{Cl^-}_{\mathrm{patient}}] \times ([\mathrm{Na^+}_{\mathrm{mean\ normal}}] / [\mathrm{Na^+}_{\mathrm{patient}}])$
II. Alterations in acid–base balance
   A. Changes in acid–base balance due to weak acids
      Δ albumin (mEq/l) $= 3.7 \times ([\mathrm{alb}_{\mathrm{mean\ normal}}]\ (\mathrm{mg/dl}) - [\mathrm{alb}_{\mathrm{patient}}]\ (\mathrm{mg/dl}))$
      Δ phosphorus:
         $[\mathrm{phos}_{\mathrm{adjusted}}]\ (\mathrm{mg/dl}) = [\mathrm{phos}_{\mathrm{mean\ normal}}]\ (\mathrm{mg/dl}) - [\mathrm{phos}_{\mathrm{patient}}]\ (\mathrm{mg/dl})$
         $\mathrm{phos}_{\mathrm{adj}}\ (\mathrm{mg/dl}) \times 0.3229 = \mathrm{phos}\ (\mathrm{mmol/l})$
         effective phos (mEq/l) $= 1.8 \times$ phos (mmol/l)
   B. Changes in acid–base balance due to alterations in [SID] (all values expressed in mEq/l)
      Δ free water $= z([\mathrm{Na^+}_{\mathrm{patient}}] - [\mathrm{Na^+}_{\mathrm{mean\ normal}}])$
         where $z = [\mathrm{SID}] / [\mathrm{Na^+}_{\mathrm{mean\ normal}}]$
      Δ chloride $= [\mathrm{Cl^-}_{\mathrm{mean\ normal}}] - [\mathrm{Cl^-}_{\mathrm{corrected}}]$
      Δ unmeasured anions (UA) = BE − (Δ free water + Δ $\mathrm{Cl^-}$ + Δ phos + Δ albumin)

BE, base excess; SID, strong ion difference; UA, unmeasured anions.

While most clinicians still favor the traditional approach to evaluation of acid–base balance, modified applications of Stewart's theories broaden this scope and lend useful quantitative insights into the complexities of acid–base disturbances (Constable, 1999).

Disorders of Acid–Base Metabolism

Disorders of acid–base equilibrium can result from a primary disturbance in pulmonary regulation of the concentration of $\mathrm{CO_2}$, from metabolic changes in strong ions and, dependently, bicarbonate, or from a combination of these mechanisms. An acid–base disturbance is considered simple if it is limited to a primary disturbance and an appropriate secondary or compensatory response. Primary disturbances and expected compensatory responses are modeled using the tail of the Henderson–Hasselbalch equation in Table 23.10 and summarized in Table 23.12. Mixed acid–base disturbances are suspected

Table 23.12 Characteristics of primary acid–base disturbances. Source: Adapted from Rose, 1994.

Disorder	pH	[H+]	Primary disturbance	Compensatory response
Metabolic acidosis	↓	↑	↓ $[HCO_3^-]$, ↓ [SID]	↓ $P\text{CO}_2$
Metabolic alkalosis	↑	↓	↑ $[HCO_3^-]$, ↑ [SID]	↑$P\text{CO}_2$
Respiratory acidosis	↓	↑	↑ $P\text{CO}_2$	↑ $[HCO_3^-]$, ↑ [SID]
Respiratory alkalosis	↑	↓	↓ $P\text{CO}_2$	↓ $[HCO_3^-]$, ↓ [SID]

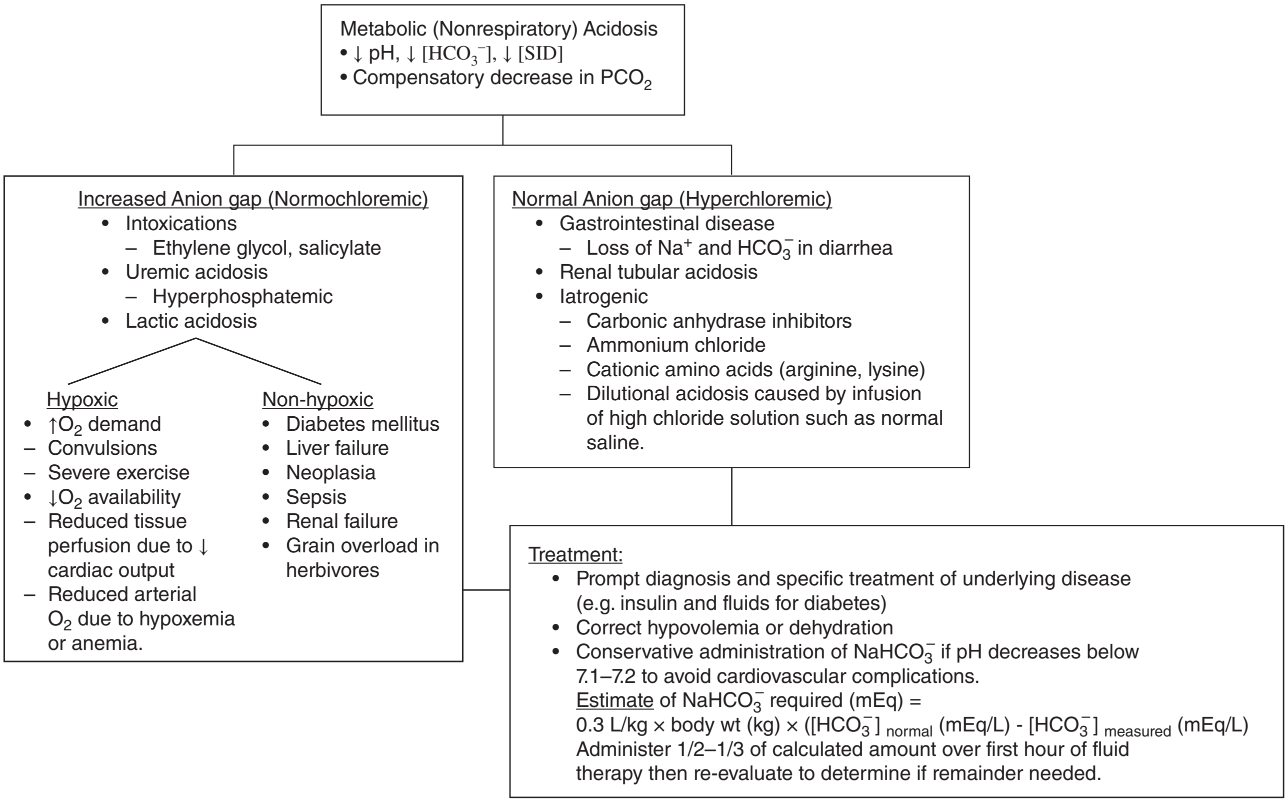
SID, strong ion difference.

when the compensatory response to a primary disorder is not as expected or when the pH is changing in a direction opposite that predicted by the primary disorder. Mixed acid–base disturbances are characterized by two or more primary disturbances in the same patient.

Metabolic (Nonrespiratory) Acidosis

Metabolic acidosis may be characterized by a decrease in plasma HCO_3^- concentration, decreased pH, increased concentration of strong anions (such as chloride, lactic acid, or ketoacids), and decreased plasma sodium concentration associated with renal disease or diarrhea. The clinical signs most commonly associated with metabolic acidosis are hyperpnea and CNS depression. Laboratory analysis of blood and urine reveals a lowered urine and blood pH, decreased serum HCO_3^- (<20 mEq/l), decreased [SID], and a variable serum $P\text{CO}_2$ depending upon the degree of respiratory compensation. Figure 23.6 summarizes causes of metabolic acidosis and provides general principles of treatment. Metabolic acidosis is the most common acid–base disorder in dogs, cats, and horses, and causes may be usefully subdivided into those conditions that increase the AG and those that do not.

Loss of Na^+ and HCO_3^- associated with diarrhea is the most common cause of normal AG (hyperchloremic) metabolic acidosis. Intestinal secretions replete in Na^+ and HCO_3^- may also be sequestered in lower obstructive bowel disease and paralytic ileus. Hypoadrenocorticism may also present with a nongap metabolic acidosis, but these patients usually have hypochloremia as a result of impaired water excretion, lack of aldosterone, and poor renal function.


Metabolic (Nonrespiratory) Acidosis
- ↓ pH, ↓ $[HCO_3^-]$, ↓ [SID]
- Compensatory decrease in PCO_2

Increased Anion gap (Normochloremic)
- Intoxications
  - Ethylene glycol, salicylate
- Uremic acidosis
  - Hyperphosphatemic
- Lactic acidosis

Hypoxic
- ↑O_2 demand
  - Convulsions
  - Severe exercise
- ↓O_2 availability
  - Reduced tissue perfusion due to ↓ cardiac output
  - Reduced arterial O_2 due to hypoxemia or anemia.

Non-hypoxic
- Diabetes mellitus
- Liver failure
- Neoplasia
- Sepsis
- Renal failure
- Grain overload in herbivores

Normal Anion gap (Hyperchloremic)
- Gastrointestinal disease
  - Loss of Na^+ and HCO_3^- in diarrhea
- Renal tubular acidosis
- Iatrogenic
  - Carbonic anhydrase inhibitors
  - Ammonium chloride
  - Cationic amino acids (arginine, lysine)
  - Dilutional acidosis caused by infusion of high chloride solution such as normal saline.

Treatment:
- Prompt diagnosis and specific treatment of underlying disease (e.g. insulin and fluids for diabetes)
- Correct hypovolemia or dehydration
- Conservative administration of $NaHCO_3^-$ if pH decreases below 7.1–7.2 to avoid cardiovascular complications.

  Estimate of $NaHCO_3^-$ required (mEq) =

  $0.3\ \text{L/kg} \times \text{body wt (kg)} \times ([HCO_3^-]_{\text{normal}}\ (\text{mEq/L}) - [HCO_3^-]_{\text{measured}}\ (\text{mEq/L})$

  Administer 1/2–1/3 of calculated amount over first hour of fluid therapy then re-evaluate to determine if remainder needed.

Figure 23.6 Causes of metabolic acidosis and general treatment principles.

Lactic Acidosis

Production of lactic acid and accumulation of lactate, an unmeasured anion, decrease the [SID], resulting in a high AG metabolic acidosis. Lactic acid is the final product of anaerobic glycolysis in eukaryotic cells and is formed by the action of lactate dehydrogenase (LDH) on pyruvic acid with NADH as a cofactor.

$$\mathrm{CH_3COO^- + NADH + H^+ CH_3CHOHCOO^-} \\ \mathrm{+ NAD^+(pyruvate)LDH(lactate)}$$

The direction of the LDH reaction depends upon the relative intracellular concentrations of pyruvate and lactate and on the ratio of reduced (NADH) to oxidized (NAD^+) nicotinamide adenine dinucleotide cofactor. Newly produced lactic acid is partially buffered by HCO_3^-, resulting in rapid generation of sodium lactate, which dissociates to lactate and sodium ions. Under aerobic conditions in the liver and the kidney, lactate is converted back to pyruvate, and pyruvate is metabolized through the tricarboxylic acid (TCA) cycle to yield HCO_3^-, CO_2, and H_2O. Alternatively, hepatic uptake of lactate and conversion to pyruvate can feed gluconeogenesis, a process that also regenerates HCO_3^-. In either case, the net result of aerobic lactate metabolism is production of alkalinizing equivalents in the form of HCO_3^-:

Conversion via the TCA cycle

$$\mathrm{lactate^- + 3O_2 \rightarrow HCO_3^- + 2CO_2 + 2H_2O}$$

Conversion via gluconeogenesis

$$\mathrm{2\ lactate^- + 2H_2O + 2CO_2 \rightarrow 2HCO_3^- + glucose}$$

If the ratio of $NADH/NAD^+$ in the cell shifts toward accumulation of NADH (e.g., in exercising muscle or poorly oxygenated tissues), more lactic acid accumulates, decreasing cellular pH. In the case of poorly oxygenated tissues, inability to oxidize NADH via the respiratory chain blocks oxidative phosphorylation and production of ATP. ATP depletion in lactic acidosis causes leaky ATP-dependent K^+ channels, leading to hyperpolarized membranes and decreased Ca^{++} influx via voltage-dependent Ca^{++} channels. Decreased intracellular Ca^{++} produces smooth muscle relaxation, vasodilation, and a potential decline in systemic blood pressure (Landry and Oliver, 1992).

Causes of the two types of lactic acidosis, hypoxic (type A) and nonhypoxic (type B), are listed in Figure 23.6. (Only L-lactate is metabolized by animals; hence the discussion that follows refers only to L-lactic acidosis and not D-lactic acidosis, a condition described in humans and associated with small bowel resection or short bowel syndrome.) Reduced tissue perfusion and hypoxia caused by cardiac arrest/cardiopulmonary resuscitation, shock, hypovolemia, left ventricular failure, low cardiac output, and acute pulmonary edema limit oxygen availability and force cells into anaerobic glycolysis. As NADH accumulates, the LDH reaction is pushed to the right, resulting in lactic acid accumulation. Successful management of most of these conditions involves returning tissue perfusion and oxygenation to normal, often with the aid of parenteral fluid administration. Reversal of circulatory failure decreases further lactate accumulation and, if the liver is well perfused, will result in conversion of accumulated lactate to HCO_3^-.

Administration of $NaHCO_3^-$ to animals suffering from lactic acidosis is controversial. Benefits could include improved tissue perfusion related to reversal of acidemia-induced vasodilation and an increase in [SID] (associated with Na^+ administration). Potential risks include overshoot metabolic alkalosis caused by the cumulative effect of $NaHCO_3^-$ administration and metabolism of the accumulated lactate into HCO_3^-. A study in rats (Halperin et al., 1996) concluded that $NaHCO_3^-$ therapy extended the period of survival during acute, hypoxic L-lactic acidosis. Hypoxia was induced in anesthetized, paralyzed rats ventilated with a lowered (5.5%) oxygen concentration, which was sufficient to cause a severe degree of L-lactic acidosis. Survival in rats receiving $NaHCO_3^-$ was close to twofold longer than in rats receiving no sodium bicarbonate or NaCl only. The rate of $NaHCO_3^-$ infusion was titrated to equal the rate of L-lactic acid appearance in the ECF of control hypoxic rats. Part of the benefit of alkali treatment was hypothesized to be increased anaerobic glycolysis, causing enhanced ATP and L-lactic acid production and a decreased oxygen consumption. Despite continued accumulation of L-lactic acid and a decrease in cardiac output that was greater than in control rats, availability of ATP for vital organs was considered critical to prolonged survival in alkali-treated animals. While results using this controlled model are not directly clinically applicable, they suggest that continued consideration of the advantages and disadvantages of alkali supplementation in L-lactic acidosis may be merited. Many clinicians favor a conservative therapeutic approach in which small amounts of $NaHCO_3^-$ are administered to keep the arterial pH above 7.1–7.2 and to avoid progressive decline in cardiovascular function (Rose, 1994). In the absence of severely elevated concentrations of lactate, and in the presence of a well-perfused liver, the use of lactate-containing alkalinizing solutions is effective for volume restoration. Alternatives to lactate-containing solutions include $NaHCO_3^-$, sodium gluconate, and sodium acetate.

Ketoacidosis and Other Causes

Metabolic acidosis associated with ketonemia and ketonuria occurs when the rate of formation of ketone bodies is greater than the rate of their use. This occurs most often in two conditions, diabetes mellitus and starvation. Excess acetyl coenzyme A (CoA) derived from fatty acid or pyruvate oxidation is diverted, primarily in the liver, to production of ketone bodies (acetoacetate, β-hydroxybutyrate, acetone). Ketones can be transported in the blood and utilized as an energy source by peripheral tissues. In diabetes the lack of insulin increases lipolysis, and an excess of glucagon indirectly increases fatty acetyl CoA entry into hepatic mitochondria for conversion to ketones. An elevation of ketones in the blood results in acidemia because the carboxyl group of the ketone body has a pK_a of about 4. At physiological pH the ketoacid is fully dissociated, losing a proton (H^+), which lowers blood pH. Addition of an unmeasured anion, the ketoacid, decreases the [SID] driving an acidosis. Ketoacidosis is often complicated by dehydration associated with osmotic (glucose-driven) diuresis. The use of alkali to treat diabetic ketoacidosis is controversial and not generally recommended. Rehydration (usually with normal saline) and administration of insulin is the treatment of choice since circulating ketoacids will subsequently be metabolized to HCO_3^- and move plasma pH toward normal.

Renal failure typically produces a normochloremic, high-AG metabolic acidosis due to accumulation of phosphates, sulfates, and other organic anions, altered handling of chloride, and an inability to excrete the daily dietary acid load. Enhanced generation of ammonia by the renal tubular cells allows the kidney to respond, up to a point, to the chronic retention of fixed acid. Use of alkali to treat metabolic acidosis associated with renal failure is controversial. Three reasons cited in support of treatment are that treatment (i) spares depletion of bone serving as a H^+ buffer, (ii) prevents the potentially catabolic effects of acidosis on muscle protein, and (iii) limits complement-mediated tubulointerstitial damage that may occur in concert with increased ammoniagenesis. Oral administration of $NaHCO_3^-$ (0.5–1.0 mEq/kg/day) with the goal of maintaining plasma HCO_3^- at 15 mEq/l may be effective if the associated sodium load does not encourage fluid retention.

Metabolic (Nonrespiratory) Alkalosis

Metabolic alkalosis is characterized by an excess of HCO_3^- caused by a deficit of H^+ in the ECF. This state may be caused by excessive vomiting (especially from gastrointestinal obstruction), excessive alkaline therapy, or use of diuretics that can create iatrogenic metabolic alkalosis, or excessive loss of potassium caused by hyperadrenocorticism or administration of large quantities of K^+-free solutions. Clinical signs of metabolic alkalosis are depressed breathing (slow and shallow), nervous excitement, including tetany, and even convulsions and muscular hypertonicity. Respiratory compensation is not as effective as respiratory compensation for metabolic acidosis.

Values for serum electrolytes usually reveal elevated [HCO_3^-], lowered [Cl^-], and variable [Na^+]. There is usually a low serum [K^+] in this condition. A relationship exists between K^+ loss and metabolic alkalosis in that each can result in the other (positive feedback). In ruminants the situation is much more complex, and unlike in small animals, metabolic alkalosis is much more common. Compensation for metabolic alkalosis requires the kidneys to excrete HCO_3^- and retain H^+. Therapy for metabolic alkalosis involves treatment of the underlying disease and, potentially, use of acidifying solutions such as NaCl (0.9%), NH_4Cl (1.9%) (NH_3^+ is conjugated to urea in the liver, which frees H^+ and Cl^-), and Ringer's solution, which supplies Na^+, K^+, Ca^{++}, and Cl^-.

Respiratory Acidosis

Respiratory acidosis (Table 23.13) involves retention of CO_2 as a consequence of alveolar hypoventilation. The fall in pH is predictable from the Henderson–Hasselbalch equation. Impaired respiration can be

Table 23.13 Causes of respiratory acidosis. Source: Adapted from DiBartola, 1992a. Reproduced with permission of Elsevier.

- Inadequate mechanical ventilation
- Airway obstruction
- Respiratory center depression
  - Neurologic disease
  - Drugs (e.g., anesthetic agents, narcotics, sedatives)
- Cardiopulmonary arrest
- Neuromuscular defects
  - Myasthenia gravis
  - Tetanus
  - Botulism
  - Polyradiculoneuritis
  - Polymyositis
  - Tick paralysis
  - Hypokalemic periodic paralysis in Burmese cats
  - Hypokalemic myopathy in cats
  - Drugs (e.g., succinylcholine, pancuronium, aminoglycosides with anesthetics, organophosphates)
- Restrictive defects
  - Diaphragmatic hernia
  - Pneumothorax
  - Pleural effusion
  - Hemothorax
  - Chest wall trauma
  - Pulmonary fibrosis
  - Pyothorax
  - Chylothorax
- Pulmonary disease
  - Respiratory distress syndrome
  - Pneumonia
  - Severe pulmonary edema
  - Diffuse metastatic disease
  - Smoke inhalation
  - Pulmonary thromboembolism
  - Chronic obstructive pulmonary disease
  - Pulmonary fibrosis

caused by pneumonia, pulmonary edema, emphysema, pneumothorax, respiratory muscle paralysis, morphine, barbiturate, or anesthetic poisoning, airway occlusion, or, most commonly, hypoventilation during positive pressure ventilation (iatrogenic). Clinical signs include respiratory distress and CNS depression with progressive disorientation, weakness, and finally coma (CO_2 narcosis). Cyanosis is often present in the advanced stages. Laboratory analysis of blood and urine will show a decreased urine pH, decreased blood pH, increased serum HCO_3^- (from tissue buffers and renal reabsorption of HCO_3^-), and a decrease in serum Cl^- because of renal excretion. Hypoventilation results in CO_2 retention, an excess of H_2CO_3, and thereby an excess of H^+. The compensatory mechanism is for the kidneys to conserve HCO_3^- and excrete H^+. The most important treatment for this condition is proper ventilation of the animal. Use of alkalinizing solutions may aid in cases of lung disease when ventilation alone will not correct the condition. Whenever possible, therapy should be directed at removal of the causative factor.

Respiratory Alkalosis

Causes of respiratory alkalosis are indicated in Table 23.14. The most common cause of this disease in animals is overactive positive pressure ventilation during anesthesia (iatrogenic). Other causes include fever, stimulation of respiratory centers by encephalitis, salicylate intoxication, a deficiency of O_2 (hypoxia), heat prostration, or conditions causing chronic hyperventilation (excessive blowing off of CO_2). Clinical signs include hyperpnea (with or without panting), hyperactive tendon reflexes, and CNS stimulation with or without convulsions. Laboratory analysis reveals increased urine pH, increased blood pH, and decreased serum HCO_3^-. Serum Cl^- is usually normal to slightly increased, and pathogenesis of the condition relates to excessive blowing off of CO_2. Compensation occurs by renal excretion of HCO_3^- and retention of H^+. Treatment for this condition should involve correcting the hyperventilation, when feasible, and use of the same acidifying solutions used for metabolic alkalosis. Underlying etiological factor(s) must be eliminated.

Table 23.14 Causes of respiratory alkalosis. Source: Adapted from DiBartola, 1992a. Reproduced with permission of Elsevier.

- Overzealous mechanical ventilation
- Hypoxemia (stimulation of peripheral chemoreceptors by decreased oxygen delivery):
  - Right-to-left shunts
  - Decreased Po_2 (e.g., high altitude)
  - Congestive heart failure
  - Severe anemia
  - Hypotension
  - Pulmonary diseases resulting in ventilation–perfusion mismatching:
  - Pneumonia
  - Pulmonary embolism
  - Pulmonary fibrosis
  - Pulmonary edema
  - Pulmonary disease resulting in stimulation of nociceptive receptors independent of hypoxemia:
  - Pneumonia
  - Pulmonary embolism
  - Interstitial lung disease
  - Pulmonary edema
- CNS-mediated hypocapnia with direct stimulation of medullary respiratory center:
  - Liver disease
  - Gram-negative sepsis
  - Drugs (e.g., salicylate intoxication, progesterone, xanthines)
  - Recovery from metabolic acidosis
  - Central neurological disease
  - Heat stroke

Mixed Acid–Base Disturbances

The preceding discussion of acidosis and alkalosis has purposely dealt with idealized, single etiological processes in the genesis of acid–base abnormalities. Such states rarely exist in real life. Mixed disturbances usually occur, and treatment will often convert one type of acid–base disturbance into another. Proper therapy must include careful appraisal of repeated laboratory determinations and close observation of the clinical situation. Using these techniques, mixed disturbances can be identified, evaluated, and managed successfully. Examples of potential causes of mixed respiratory and metabolic disorders are noted in Table 23.15.

Practical Aspects of Fluid Therapy

Diagnosis and Monitoring

When fluid therapy is under consideration, the practitioner must ask the following six questions: (i) When should fluid therapy be instituted? (ii) What kind(s) of solution(s) should be used? (iii) How much fluid should be administered? (iv) How fast should the solution be given? (v) What route of administration should be used? (vi) How will the success of the therapy be evaluated? The answers to these questions are individual in nature and are critically dependent on a knowledge and understanding of normal homeostatic mechanisms. They are also dependent on the history of the patient, a basic understanding of how a particular disease affects water and electrolyte balance, and a correct diagnosis.

The purpose of fluid and electrolyte therapy is to correct dehydration or overhydration and electrolyte imbalance and/or acid–base imbalance. It may also be indicated to correct a condition of acidosis or alkalosis,

Table 23.15 Examples of potential causes of mixed respiratory and metabolic disorders. Source: Adapted from DiBartola, 1992a. Reproduced with permission of Elsevier.

- Respiratory acidosis and metabolic acidosis
  - Hypoadrenocorticism-like syndrome in dogs with gastrointestinal disease
  - Cardiopulmonary arrest
  - Severe pulmonary edema
  - Thoracic trauma with hypovolemic shock
  - Low-cardiac-output heart failure with pulmonary edema
  - Advanced septic shock
  - Gastric dilatation volvulus
  - Acute tumor lysis syndrome
- Respiratory acidosis and metabolic alkalosis
  - Pulmonary edema and diuretics
  - Gastric dilatation volvulus
- Respiratory alkalosis and metabolic acidosis
  - Hypoadrenocorticism-like syndrome in dogs with gastrointestinal disease
  - Septic shock
  - Salicylate toxicity
  - Heat stroke
  - Gastric dilatation volvulus
  - Liver disease (renal tubular acidosis and impaired metabolism of lactate)
  - Lactic acidosis with excessive hyperventilation
  - Pulmonary edema
  - Parvovirus gastroenteritis and septicemia
  - Severe exercise
  - Acute tumor lysis syndrome
  - Cardiopulmonary resuscitation
- Respiratory alkalosis and metabolic alkalosis
  - Gastric dilatation volvulus
  - Hyperadrenocorticism with pulmonary thromboembolism
  - Ventilator-induced mixed alkalosis (too rapid correction of abnormal arterial $P\text{CO}_2$)
  - Congestive heart failure and diuretics
  - Hepatic disease and diuretics
  - Vomiting or hypoproteinemia
  - Parvovirus gastroenteritis and septicemia

treat shock, give parenteral nourishment, or even stimulate organ function (i.e., the kidneys). Causes of fluid, electrolyte, and/or protein loss include situations wherein substances are not available because of lack of supply or condition of the animal; for example, an animal with a fractured mandible may be unable to take in food or liquid, or an animal with a CNS disturbance may be unable to eat or drink because of the primary disease state. Other causes of fluid, electrolyte, and/or protein imbalances may involve excessive elimination.

The following information must be acquired by questioning the owner, observation of the patient, and/or clinical examination: duration and frequency of vomiting and/or diarrhea, consistency of stools, frequency of urination, color of urine, presence and character of thirst, fluid and dietary intake, dryness or elasticity (turgor) of the skin, nature and color of the mucous membranes and sclera, presence of excessive salivation or panting, odor of the breath, and weight loss or gain.

In combination with clinical signs, laboratory examination of the blood provides a rational basis for estimating patient fluid and electrolyte needs and monitoring treatment success. Measurements should include hematocrit, plasma protein, blood gases ($P\text{O}_2$, $P\text{CO}_2$, base excess, HCO_3^-, or total CO_2) and electrolytes (Na^+, K^+, Cl^-), blood urea nitrogen, and creatinine. Because red blood cells and plasma protein are largely limited to the vascular space, the concentration of both tends to increase with dehydration. It is best to assess both hematocrit and plasma protein since results of one or the other test alone can be misleading if preillness values are out of the normal range. For example, preexisting anemia, hypoproteinemia, or physiological events such as splenic contraction can confound interpretation of either parameter if considered alone.

Collection, measurement, and analysis of urine are important for proper care of the critically ill patient. Urinalysis should include tests for specific gravity, glucose, acetone, pH, and albumin and microscopic sediment examination. During a state of dehydration, if the kidneys are functioning normally, specific gravity will increase and urine volume will decrease. If the specific gravity of urine is unchanged or lowered and the animal shows clinical signs of dehydration, the kidneys are probably not functioning properly, and more sophisticated renal function tests must be employed. Specific gravity of urine should be monitored during the treatment period. A decrease in this parameter indicates that hydration is taking place. If the animal has not yet received treatment with a solution containing glucose and it is found in the urine, diabetic acidosis is possibly the cause of dehydration. The urine glucose should also be monitored during treatment. If the animal is receiving glucose and the urine glucose reaches $^+3$ or $^+4$, the dosage must be lowered. Acetone in the urine is a frequent finding during dehydration and/or carbohydrate starvation. If the pH of the urine in species with normally acid urine tests alkaline, a diagnosis of alkalosis may be indicated if no kidney or urinary tract disease is present. The presence of urinary albumin and sediment may be an indication of renal disease. If the kidneys are functioning properly, they can adjust markedly to insult. However, in the presence of renal impairment, therapy must be specific or the treatment may be fatal.

Diligent assessment of clinical signs and laboratory parameters is essential to successful diagnosis and monitoring of fluid and electrolyte imbalances. Useful parameters are summarized in Table 23.16.

Fluid Volume and Type

A standard approach to estimating fluid volume needs should be used. Replacement of adequate volume is often the single most important key to improved clinical status

Table 23.16 Parameters to be monitored during fluid therapy. Source: Adapted from DiBartola, 1992a. Reproduced with permission of Elsevier.

- Normal bronchovesicular lung sounds on auscultation
  - Packed-cell volume
  - Total protein
  - Electrolytes: Na^+, Cl^-, Ca^{2+}, HCO_3^-
  - Arterial pH
  - Arterial $P\text{CO}_2$
  - Urine output
  - Body weight
  - Hemodynamics
    - Central venous pressure
    - Pulmonary capillary wedge pressures
    - Mean arterial pressure
    - Mean pulmonary arterial pressure

of animals with multiple fluid and electrolyte disturbances. Volume replacement should have three specific aims: correct existing deficits, satisfy maintenance needs, and replace continuing loss. Initial volume deficits are addressed by administration of replacement fluids. Calculation of the amount of fluid needed is based on clinical and laboratory assessment of percent of dehydration. See Table 23.5 for a summary of signs correlated to degree of dehydration. The volume needed to address the initial deficit is estimated according to the following equation:

$$\text{Replacement volume (l)} = \text{body weight (kg)} \times \%\ \text{dehydration}$$

Clinicians working with both small and large animals should become comfortable with the large differences in volume that will be required to address deficits in different animals. For example, the replacement volume needed to address an 8% fluid deficit in a dehydrated mare weighing 500 kg is 100 times greater than that needed for a similarly dehydrated cat weighing 5 kg. Forty liters of fluid would initially be administered to the mare versus 400 ml to the cat. In general, the composition of replacement fluids should reflect the composition of the volume of fluid lost. For example, if the volume deficit is related to loss of electrolyte-rich gastrointestinal fluid, then a balanced replacement solution containing Na^+, K^+, Cl^-, and bicarbonate equivalents would likely be selected. Table 23.17 details the compositions of commonly utilized replacement fluids.

Table 23.17 Composition of selected fluid therapy solutions

		Characteristics		Ion composition (mEq/l)						
Type	**Solution**	**pH**	**Osmolarity (mOsm/l)**	**Na^+**	**K^+**	**Cl^-**	**Ca^{++}**	**Mg^{++}**	**Glucose (g/l)**	**Alkalinizing equivalents (mEq/l)**
Replacement										
Acidifying BES	Ringer's	5.4	309	147	4	155	4	0	0	0
Acidifying BES	Normal saline (0.9%)	5.0	308	154	0	154	0	0	0	0
Alkalinizing BES	Lactated Ringer's	6.6	273	130	4	109	3	0	0	28 (lactate)
Alkalinizing BES	Normosol-R	6.6	294	140	5	98	0	3	0	27 (acetate) 23 (gluconate)
Alkalinizing BES	Plasma-Lyte A	7.4	294	140	5	98	0	3	0	27 (acetate) 23 (gluconate)
Maintenance										
Acidifying	2.5% dextrose/water in 0.45% saline plus potassium addition (16 mEq/l)	4.5	280	77	16	77	0	0	25	0
	Equal volumes 5% dextrose/water and lactated Ringer's plus potassium addition (16 mEq/l)	5.0	309	65.5	18	55	1.5	0	25	14 (lactate)
	Normosol-M with 5% dextrose	5.0	363	40	13	40	0	3	50	16 (acetate)
	Plasma-Lyte M with 5% dextrose	5.5	377	40	16	40	5	3	50	12 (lactate) 12 (acetate)
Other solutions	5% dextrose/water	4.0	252	0	0	0	0	0	5	0
	50% dextrose/water	4.2	2780	0	0	0	0	0	50	0
	7.5% saline	—	2566	1283	0	1283	0	0	0	0
	8.4% $NaHCO_3$	—	2000	1000	0	0	0	0	0	1000
	14.9% KCl	—	4000	0	2000	2000	0	0	0	0

BES, balanced electrolyte solution.

In addition to replacing existing deficits, maintenance fluid needs must be calculated. Maintenance fluids are needed when a patient does not voluntarily ingest sufficient food and water to replace normal losses occurring via urine, feces, respiratory tract, and skin. The average resting animal at standard conditions of humidity and temperature has a rather constant rate of water turnover. For practical purposes, 40–65 ml/kg/24 h (30 ml/lb/day is often used as a rule of thumb) for mature animals and 130 ml/kg/24 h for immature animals serve as average water turnovers for all mammalian species. Based on these assumptions, an average mature dog weighing 20 kg requires about 1.3 l for a daily maintenance supply of water, while a horse weighing 450 kg would require about 29 l/day. Maintenance needs may be modified under conditions of severe stress or fever, extreme environmental conditions, or in the presence of various disease processes. Older animals may need more or less maintenance volume depending upon the presence of polyuria or compromised cardiovascular function, respectively. Administration of various drugs (e.g., glucocorticoids, diuretics) will also affect maintenance needs. The electrolyte composition of fluids used for maintenance differs from that of replacement fluids used to address initial deficits. Because of the composition of fluid lost daily in urine and as insensible loss from the skin and respiratory tract, maintenance fluids are typically lower in sodium (approximately 40 mEq/l) and higher in potassium (approximately 10–16 mEq/l) than replacement fluids. Table 23.17 details the composition of both commercial maintenance fluids and maintenance fluids that can be prepared using other commonly available fluid components. In veterinary medicine, in contrast to human medicine, patients are less often transitioned from replacement to maintenance fluids. Consequently, veterinary hospitals do not typically stock commercially available maintenance fluid.

If the animal being treated continues to lose water during the treatment period (e.g., due to continued vomiting, diarrhea, polyuria) this additional amount must be estimated and added to the replacement and maintenance volumes. The volume required to replace continued loss is based on clinical observation (e.g., frequency of defecation, character and volume of feces in the case of diarrhea). Like the volume used to address the initial deficit, the type of fluid selected to replace continuing loss should, in general, resemble the fluid lost. More often than not, balanced electrolyte solutions such as lactated Ringer's are chosen.

Application of the principles outlined above may be appreciated using the following case example. A 2-year-old, 20-kg mixed-breed dog presents with a chief complaint of diarrhea of 2 days' duration. A physical exam reveals a loss of skin elasticity and a definite delay in return of skin to normal position when tented. Both mucous membranes and tongue are dry and the eyeballs feel soft and slightly sunken. Capillary refill time is slightly prolonged. Based on these clinical signs, dehydration is assessed at 8%. The dog is continuing to pass semifluid stools every 2–3 hours, resulting in an estimated ongoing loss of 150 ml/day. The owner reports that the dog is not eating or drinking. Calculation of the volume of fluid to be administered to this dog over the next 24 hours would include

Replacement of initial deficit:	20 kg × 0.08 =	1.6 l
Maintenance needs:	65 ml/kg/day × 20 kg =	1.3 l
Continued loss:		0.15 l
Total estimated fluid needs:		3.05 l

This volume is considered an estimate because it is based on clinical signs and average maintenance losses. Despite the importance of good data collection and appropriate application of fluid therapy principles, at some level adjusting volume is dependent upon a "guess and reassess" process driven by diligent and thorough patient observation (Roussel, 1990).

Rates and Routes of Administration

The rate of fluid and/or electrolyte replacement should parallel the severity of dehydration and electrolyte or acid–base imbalance. Fluids should be administered rapidly at first and then at decreasing rates until the condition is corrected. The rate of infusion is slowed after the first hours of administration to align with a daily administration rate of 200 ml/kg/day or about 8 ml/kg/h. Rates of about 15 ml/kg/h are cited and Cornelius et al. (1978) have shown that rates of 90 ml/kg/h are tolerated in moderately dehydrated, unanesthetized normal dogs. These rates of fluid administration are not recommended for ill animals (e.g., cardiac, renal or other dysfunction) as heart failure can occur with overly aggressive fluid administration. No deaths occurred in the Cornelius study, but clinical signs of severe overhydration were evident in dogs given fluids at 360 ml/kg/h. At 90 ml/kg/h pulmonary artery wedge pressures and central venous pressures were increased in dogs with normally functioning hearts. A seriously ill dog, with compromised cardiac muscle contractility, could be injured by infusion rates that result in acute volume overload. If central venous pressures are being monitored, the infusion rate can be individually adjusted for each patient. This technique is simple and inexpensive. The attending veterinarian should monitor this parameter in the critically ill patient and adjust the rate of fluid administration according to individual needs.

Infusion rates of 50 ml/kg/h have been tolerated in severely dehydrated cases but this is considered aggressive. Less severe cases could be aggressively managed with initial rates of 15–30 ml/kg/h but lower rates are often used. In all cases the rate of infusion should be slowed after the first hour of administration and should be slowed considerably if no urine flow is established. After 4 or more hours of fluid administration without urine flow, the rate of administration should be 2 ml/kg/h or less. Every attempt must be made to establish renal function if no urine flow is detected after 2 hours of fluid administration. To accurately monitor urine flow, all critically ill animals should have a urinary bladder catheter in place.

Common sense and clinical judgment must be exercised. If an animal is severely dehydrated and in shock, it is difficult to administer fluids too fast during the initial stages of treatment. If, however, an animal is almost normally hydrated and the aim is only to maintain hydration, the rate should be slowed considerably. The importance of renal function has been repeatedly emphasized and is typically assessed by measurement of blood urea nitrogen, creatinine, and urine specific gravity.

The route of fluid administration depends on the type of illness being dealt with and the severity of the condition, degree of dehydration, condition of the patient, type of electrolyte imbalance, organic functions of the patient, and time and equipment available. Probably the easiest, most physiological, and most overlooked route of administration of fluid and electrolytes is oral or nasogastric. The oral route is the least dangerous, since the solution can be administered without strict attention to tonicity, volume, and asepsis. Oral replacement of electrolytes by using combinations of electrolyte salts, glycine, and dextrose has been especially successful (Hamm and Hicks, 1975). Proper technique for oral fluid administration should preclude complications associated with fluid aspiration or administration of excessive amounts of air. A relatively unused route of administration that might be considered, especially in very young animals, is per rectum. Warm water, K^+, Na^+, and Cl^- are well absorbed via this route. It may be difficult, however, to get the animal to retain material given in this manner, especially in the presence of gastrointestinal disease. Rectal infusion of fluids in birds has been suggested as an effective alternative route to intravenous, intraosseous, oral, or subcutaneous (Ephrati and Lemeij, 1997).

The most commonly used and perhaps most practical routes of fluid and electrolyte administration are the parenteral routes: intravenous (IV), subcutaneous (SC), intraperitoneal (IP), or intraosseous (IO). The IV route is the most versatile. Severe disturbances of fluid and electrolyte balance demand it. Nearly all the toxicity of solutions administered in this manner is more related to rate than volume or composition. No indications for hypotonic solutions have been found, but indications for isotonic and hypertonic solutions exist, and some of these have been discussed previously. Some of the problems associated with IV administration include those associated with maintenance and asepsis of indwelling catheters, clotting, and hematomas, as well as the location of a vein on very small or very ill animals. Obviously, the fluids administered and equipment used must be sterile. Large volumes of fluid administered too rapidly may overload the circulatory system, causing pulmonary edema and even death, especially in severely ill or toxic cases. This is the preferred route for blood, blood plasma, and plasma volume expanders.

Subcutaneous administration of fluid is referred to as hypodermoclysis. This technique is convenient for correction of mild to moderate deficits in small animals. Fluids are absorbed more slowly than by the IV route, but if the animal is not in critical condition, this is of no real consequence. Only isotonic solutions should be used in this manner. Dextrose of any tonicity or any solutions lacking electrolytes in isotonic levels are contraindicated because they may produce an initial rapid diffusion of major extracellular electrolytes to the area. This can result in severe reactions, including death, especially if the animal is already in shock. Hypodermoclysis is extremely valuable in very young or very small animals. If the animal is difficult to restrain long enough for a prolonged IV infusion, this is a useful technique. When edema is present, absorption will not occur, and this route of administration is contraindicated. If the animal is chilled by a cold environment or a cold fluid is injected, absorption by this route will be delayed, and it is recommended that fluids be prewarmed to body temperature when feasible. Administration of fluids in one anatomical location should be limited to amounts that are readily absorbed (approximately 10–12 ml/kg) (Greco, 1998). Fluid should be deposited dorsally along the area bordered by the scapulae anteriorly and the iliac crests posteriorly. Hypodermoclysis is not commonly used as a route of administration in large animals.

IP infusion of fluids has the same restrictions as those for hypodermoclysis. The technique may predispose to peritonitis, so aseptic procedures must be used. The fluids are mobilized faster than in SC administration, but this route is potentially more hazardous (puncture of abdominal organs). Nevertheless, this is a good route for electrolyte and water absorption. Plasma and a large percentage of red blood cells administered using this technique are rapidly absorbed. In large animals it can be a very practical method of treatment, since a large quantity of fluid can be administered rapidly with few adverse effects. Perhaps the greatest application of this technique is with peritoneal lavage.

Intraosseous fluids are administered through a catheter placed into bone. This route may be chosen if vascular access is limited or cannot be performed in adequate time due to very small patient size, cardiac arrest,

Table 23.18 Indications, dosages, administration, and side effects associated with use of selected colloids in dogs. Source: Modified from Rudloff and Kirby, 1998. Other sources for information in table include Mathews, 1998 and Hughes, 2000.

Type of colloid	Indications	Dosage and administration	Side effects and contraindications
Plasma	Coagulopathies; disseminated intravascular coagulation; low antithrombin; acute hypoalbuminemia.	20–30 ml/kg/day administered: (a) continuously over 24 h, (b) as a 2–4 h infusion, (c) 6–10 ml/kg in 1-h infusions every 8 h, or (d) until plasma albumin is over 2.0 g/dl. Approximately 22.5 ml/kg of plasma needed to increase patient albumin by 5 g/l.	Rapid volume expansion may be detrimental to patients with oliguric or anuric renal failure or congestive heart failure.
Dextran 40	Rapid, short-term intravascular volume resuscitation from hypovolemic shock; rapid improvement of microcirculatory flow by lowering blood viscosity; prophylaxis of deep vein thrombosis and pulmonary emboli.	10–20 ml/kg/day IV bolus to effect; with distributive shock due to SIRS dextran can be followed by a CRI of hetastarch to maintain MAP of at least 80 mm Hg.	*See* plasma. Dilutional effect on serum coagulation factors in addition to possible direct effects on these factors. Contraindicated in patients with severe coagulopathies. Sludging of RBCs in microcirculation in dehydrated patients may occur if sufficient crystalloids are not administered. Anaphylaxis reported in humans. AKI has been reported.
Dextran 70	Rapid, intravascular volume resuscitation from hypovolemic, traumatic, or hemorrhagic shock.	*See* dextran 40.	*See* dextran 40. Dextran 70 is thought to impair coagulation more than dextran 40. No AKI reported.
Hetastarch (hydroxyethyl starch or HES)	Rapid, intravascular volume resuscitation from all forms of shock; small-volume resuscitation; volume replacement and maintenance in SIRS patients.	10–40 ml/kg/day IV bolus to effect; with cardiogenic shock, pulmonary contusions, or head injury, 5 ml/kg boluses are administered to effect, using the smallest volume possible to maintain MAP of 80 mm Hg.	*See* dextran 40. Anaphylaxis has not been reported with hetastarch, but pruritus possibly associated with deposits of HES in cutaneous nerves has been reported in up to 33% of patients treated with long-term infusions. AKI may be a concern.
Pentastarch (PEN)	Rapid, intravascular volume resuscitation from hypovolemic, traumatic, or hemorrhagic shock.	10–25 ml/kg/day; terminal half-life shorter than HES.	*See* dextran 40. Anaphylaxis and AKI have not been reported.
Vetstarch	Treatment and prophylaxis of hypovolemia. Not a substitute for red blood cells or coagulation factors in plasma	Up to 20 ml/kg/day in small animal patients; infuse initial 10–20 ml slowly and observe for possible anaphylactoid reactions.	See dextran 40.

AKI, acute kidney injury; CRI, constant-rate infusion; MAP, mean arterial pressure; SIRS, systemic inflammatory response syndrome.

hypovolemic shock, or patient anatomy (Mazzaferro, 2009).

Products for Fluid Therapy

Major categories of parenteral fluids include crystalloids, colloids, blood replacements, and nutritional solutions. Blood replacement products (whole blood, blood components, and red blood cell substitutes) and nutritional solutions (amino acids and fat emulsions) are considered elsewhere. The composition and characteristics of selected crystalloid solutions and additives used to spike parenteral solutions are listed in Table 23.17. Types and recommended dosages of synthetic colloids are listed in Table 23.18.

Crystalloids

As detailed in Table 23.17, crystalloid solutions are polyionic but differ in the amount of each ion and in tonicity. As discussed previously, the tonicity of parenteral fluids partially dictates distribution of volume into interstitial and intracellular spaces. Fluids that most closely resemble the ECF are isotonic, high in sodium, and low in potassium and may be acidifying or alkalinizing. These replacement fluids, also referred to as balanced electrolyte solutions (BES), may be given in large volumes at a rapid rate to patients in shock in an attempt to reestablish effective perfusion without severely altering electrolyte concentrations. Alkalinizing solutions depend upon metabolism of various substrates (e.g., lactate, acetate, gluconate) to alkalinizing equivalents in order to reduce acidemia. Lactate and acetate are metabolized in the liver and muscle, respectively, while gluconate is metabolized widely in the body. Perfusion and function of the liver are required for generation of alkalinizing equivalents from the most commonly used replacement fluid, lactated Ringer's solution. A large percentage of veterinary patients that require fluid therapy suffer from nonrespiratory acidosis and are treated with alkalinizing balanced electrolyte solutions. These fluids are generally indicated for animals suffering from diarrhea, vomiting (assuming vomitus contains bile), renal disease, trauma, and shock and those requiring pre- and postsurgical support. To avoid calcium precipitation, calcium-containing balanced electrolyte solutions, such as lactated Ringer's solution, should not be coadministered through the same port with whole blood or sodium bicarbonate.

Normal saline and Ringer's solution are considered acidifying solutions and are used to treat the relatively small percentage of small-animal patients that present with metabolic alkalosis. Both solutions are high in chloride and promote renal excretion of bicarbonate. Normal saline is also commonly used in treatment of patients with electrolyte disorders such as hyperkalemia or hypercalcemia in which absence of electrolytes in parenteral fluids is desirable. Assuming appropriate insulin therapy is instituted, normal saline is also considered the fluid of choice for treatment of diabetic ketoacidosis.

Colloids

The critical distribution of water between plasma and interstitial fluid is maintained in part by the colloid osmotic pressure (COP) of plasma protein. COP includes the osmotic pressure exerted by plasma proteins and their associated electrolyte molecules. This force draws water into capillaries and balances the hydrostatic pressure driving water out (see Starling relationships described in Section Fluid and Electrolyte Distribution). Although the basic concept of Starling relationships is straightforward, in vivo application of these concepts is complicated by the heterogeneity of Starling forces within different tissues and the complexity of transvascular fluid dynamics. Despite these caveats, it is practical to say that the balance between intravascular COP and capillary hydrostatic pressure drives net fluid extravasation and forms the basis for intravenous colloid therapy.

Therapeutic colloids may be of two types: natural and synthetic. Natural colloids include whole blood, plasma, and albumin. Synthetic colloids, include dextran 40, dextran 70, hetastarch, pentastarch, and oxypolygelatin. Therapeutic colloid solutions contain large particles and are retained within the vascular space more readily than crystalloids. As a result, smaller volumes of colloids cause greater volume expansion than crystalloids do. Initial tissue perfusion has been found to be better after volume expansion with colloids or combinations of colloids and crystalloids than with crystalloids alone (Funk and Baldinger, 1995). The duration of this effect varies and is dependent upon many variables, including the species of animal, dose, specific colloid formulation, preinfusion intravascular volume status, and microvascular permeability (Hughes, 2000).

The osmotic effect of colloid solutions is related to the number of particles rather than the size of particles in a solution. However, heterogeneity of particle size causes considerable complexity in the pharmacokinetics of these solutions. Synthetic colloids contain molecules that vary in molecular weight more than the molecules in a solution of a natural colloid such as albumin. After synthetic colloids are administered, the smaller molecules pass rapidly into the urine and are eliminated or move to the interstitium, negating their ability to attract water into the vasculature. Larger molecules remain in the circulation to exert COP until they are hydrolyzed by amylase or removed by the monocyte phagocytic system. Because of differences in particle behavior and in pharmacokinetic study design (e.g., duration of study, volume status of study subjects, volumes and rates of colloid administration), specific half-lives reported for colloids may vary considerably (Mathews, 1998). Such variation may pose therapeutic problems since actual duration of action of colloids may not coincide with manufacturer estimates of the same.

Indications for colloid use include perfusion deficits, hypooncotic states, deficiency of blood components, and diseases that lead to systemic inflammatory response syndrome (SIRS). SIRS is a generalized inflammatory process with evidence of decreased organ perfusion. Sepsis may be the source of SIRS but other conditions may also result in generalized systemic pathophysiology (e.g., heat stroke, acute pancreatitis, and neoplasia). Hallmarks of SIRS include alterations in temperature, heart rate, respiratory rate, $P\text{CO}_2$, and white blood cell count. Peripheral vasculature dilates, capillary permeability increases, and plasma proteins leak from affected vessels. The resulting hypoalbuminemia leads to a reduction in COP, loss of vascular volume, and hypoperfusion of tissues. High-molecular-weight colloids administered to SIRS patients are retained more effectively in leaky vessels and force retention of volume. Approximately 20–24% of crystalloid remains within the vasculature 1 hour after infusion into normal animals compared with 100% of the volume of infused colloid. Hence, colloids may initially expand the volume of the intravascular space approximately fourfold more than crystalloids (Hughes, 2000).

Colloids are often included in fluid regimens for small-volume resuscitation (e.g., during traumatic, hypovolemic, or cardiogenic shock), improvement of microcirculatory flow and capillary integrity (e.g., SIRS), and management of ongoing hemorrhage. While colloids are useful in reestablishing vascular integrity, replenishment of interstitial and intracellular fluid deficits depends upon appropriate use of colloids and crystalloids in combination. Colloid administration typically reduces the required amount of crystalloid fluid by as much as 40–60% (Rudloff and Kirby, 1998). Care must be taken to adjust amounts and rates of all fluids administered to prevent intravascular volume overload and subsequent interstitial edema. Monitoring of colloid therapy ideally includes direct measurement of COP with a membrane osmometer in addition to measurement of traditional indices of perfusion and hydration.

Problems associated with colloid therapy may include dilutional effects caused by expansion of the intravascular space. Packed-cell volume, albumin concentration, serum potassium concentration, and amount of circulating coagulation factors typically decline following administration of synthetic colloids. Rapid volume expansion may be of greatest concern in patients with oliguric or anuric renal failure or congestive heart failure. Precipitation of acute renal failure (now more commonly referred to as acute kidney injury) has been reported in humans with dextran 40 (Ferraboli et al., 1997) and is also of concern in veterinary patients (Hayes et al., 2016). Impairment of coagulation as a result of dilution of coagulation factors is another clinically important potential side effect (Gauthier et al., 2015). Adverse effects and lack of outcomes advantages of resuscitation using colloids as compared to crystalloid solutions has been shown in humans and is of concern in veterinary patients (Cazzolli and Prittie, 2015). Anaphylactic or anaphylactoid reactions associated with colloids have been reported in humans. Concern has also been raised over the effects of selected colloids on reticuloendothelial function (Hughes, 2000). Because cats are more likely to show signs of allergic reactions, especially when synthetic colloids are administered quickly, only small volumes infused at slow rates (5 ml/kg increments given over 5–10 minutes, repeated to effect up to 20 ml/kg) are recommended for use in this species.

Table 23.18 lists indications, dosages, and administration details for colloids used to treat dogs. Albumin (66,000–69,000 daltons) accounts for 80% of the COP of the only natural colloid listed, plasma. Each gram of albumin can retain as much as 18 ml of fluid in the intravascular space, assuming infused albumin does not leak from damaged vessels. The intravascular half-life of albumin in plasma is approximately 16 hours (Mathews, 1998). The three major categories of synthetic colloids are dextrans, hydroxyethyl starches, and gelatins. Dextrans are prepared from a macromolecular polysaccharide produced by bacterial fermentation of sucrose. Because these products represent a range of molecules with different molecular weights, they are described by a weight average molecular weight (MWw). MWw is defined as the sum of the number of molecules at each molecular weight times their mass divided by the total weight of the molecules. Dextran 70 (MWw = 70,000 daltons) was used in the past as a 6% solution in either 0.9% saline or 5.0% dextrose but is no longer readily available. Hydroxyethyl starches are derived from plant amylopectin and are modified by hydroxyethylation to reduce hydrolysis by amylase. Hetastarch (Hespan®) has a MWw of 100,000–300,000 daltons and is available as a 6% (6 g/dl) solution in 0.9% saline. Vetstarch (Voluven®)(6% hydroxyethyl starch 130/0.4 in 0.9% sodium chloride) is increasingly preferred due to fewer side effects. Pentastarch has a narrower range of molecular weights and a shorter duration of action than hetastarch. In the United States pentastarch is only approved for leukapheresis, while in Canada it is approved as a plasma volume expander. In 2013, the FDA announced a boxed warning on increased mortality, renal injury, and risk of bleeding with use of hydroxyethyl starch solutions in some settings with human patients.

Hypertonic Solutions

For several decades, resuscitation of experimental and clinical animals suffering from shock has been attempted using hypertonic saline (HSS), and studies have generally supported the benefits of HSS for transient restoration of cardiovascular function. Although a full understanding of the mechanism of action has been elusive, there is agreement that the primary benefits of HSS infusion result from plasma volume expansion. High circulating concentrations of sodium attract water into the vasculature from the interstitial and intracellular spaces and help to restore capillary flow and tissue perfusion. Cardiac output has been reported to increase as a result of increased preload, decreased afterload related to systemic and pulmonary vasodilation (Constable et al., 1995), increased adrenergic activity through release of catecholamines, and improved oxygen delivery to the heart (Tobias et al., 1993). Positive inotropy has also been reported but this remains a controversial point (Cambier et al., 1997). In vitro studies have shown that, at least during the initial treatment period, negative inotropy may predominate (Constable et al., 1994). All of the above effects are short lived (peak occurs within approximately 1 hour) but resuscitative benefits may be prolonged by combination of HSS with colloids such as dextran 70. Ideally, rapid recovery of cardiovascular parameters occurs with administration of smaller volumes of HSS or HSS plus dextran (HSD) compared to crystalloids, thus decreasing the risk of edema related to volume overload. In addition to primary volume expansion, HSS is thought to invoke a lung vagal reflex important to circulatory control during hypovolemia. How much this reflex contributes to the cardiovascular effects of HSS infusion remains controversial. HSS may also have immunomodulatory effects that protect organs from oxidative injury and enhance cell-mediated immunity (Coimbra et al., 1996).

HSS use is indicated in the treatment of shock associated with hemorrhage (Bauer et al., 1993), trauma (Schertel et al., 1996), gastric-dilatation volvulus (Schertel et al., 1997), acute pancreatitis (Horton et al., 1989), burns (Horton et al., 1990), and sepsis (Fantoni et al., 1999; Maciel et al., 1998). The evidence for use of HSS in the first three of these is most compelling, with fewer studies unequivocally demonstrating advantage under specific study conditions associated with the other disorders. HSS has also been utilized in treatment of head injury since, like mannitol, HSS draws interstitial and intracellular water away from edematous tissues and into the vasculature (Prough and Zornow, 1998). Regardless of the indication, HSS effects are transient, necessitating combination with crystalloids or colloids to achieve long-term resuscitative goals. Effects of HSS should be monitored by improvement in cardiovascular parameters correlated with increased perfusion as well as by assessment of mean arterial blood pressure, electrocardiogram, and electrolytes. Monitoring is aimed at preventing volume overload and electrolyte imbalances that may occur as a result of therapy.

HSS use is contraindicated in hypernatremic patients or those with increased plasma osmolality. Use in dehydrated animals is controversial since these patients frequently suffer from increases in both parameters. Studies that support HSS use in the presence of dehydration include those in which resuscitation with HSD of hypovolemic, diarrheic calves found this method to be at least as effective as others (Constable et al., 1996; Walker et al., 1998). In animals suffering from shock related to trauma and hemorrhage, two additional problems, hypokalemia and increased risk of rehemorrhaging, may be of concern. Rehemorrhage – bleeding caused by breakdown of clots in areas where hemorrhage has previously occurred – may be related to the sudden increase in cardiac output and arterial blood pressure associated with HSS resuscitation (Schertel and Tobias, 2000). HSS may also dilute circulating coagulation factors and affect platelet function. As with colloids, these concerns may only be of practical significance if the patient suffers from preexisting coagulopathies or thrombocytopenia. Using a swine model of hemorrhagic shock, Dubick et al. (1993) demonstrated that the combination of 7.5% NaCl/6% dextran 70 did not significantly affect various measures of coagulation and platelet aggregation in their model. Studies continue to address the pros and cons of HSS use in various animal models and in clinical patients (Krausz, 1995). Variation across species lines, differences in physiological circumstances of each study, and different views of cost versus benefit ratios may account for differing conclusions on the overall value of HSS treatment.

HSS is administered most effectively in combination with colloids or crystalloids in order to optimize resuscitative effects; 5% HSS, at a dose of 6–10 ml/kg, and 7–7.5% HSS, at a dose of 4–8 ml/kg, are administered at a rate of 1 ml/kg/min. Similar dosages may be used for HSD. More rapid administration rates may invoke a vagal-mediated hypotension, decreased heart rate, bronchoconstriction, and rapid, shallow breathing. To prepare 7% saline in 6% dextran 70, 33.0 g of anhydrous sodium chloride is added to a 500 ml bag of 6% dextran 70 in 0.9% saline. Half of the sodium chloride crystals are placed into the barrel of a 35 ml syringe and an adequate volume of dextran 70 solution is drawn into the syringe to dissolve the crystals. This solution is filtered through a 0.22 μm filter and is injected back into the bag of dextran 70. The procedure is repeated a second time to dissolve the remaining half of the sodium chloride (Schertel and Tobias, 2000). Although a reported advantage of HSS is presumed sterility due to hypertonicity, St. Jean et al. (1997) have demonstrated the ability of bacteria to adapt and survive in the hypertonic environment of HSS. Hence, aseptic technique consistent with handling of all intravenous fluids should be followed.

Blood Substitutes

The term *oxygen carrier* (or *oxygen therapeutic*) has replaced the terms *blood substitute* and *red blood cell substitute* because it more appropriately describes the common oxygen-carrying role of these agents, which have some but not all of the functions of blood (Wohl and Cotter, 1995; Awasthi, 2005). Two types of products have been developed for use as oxygen carriers—perfluorocarbons (PFCs) and hemoglobin-based oxygen carriers (HBOCs). Neither of these two types of products are currently readily available.

Perfluorochemicals

Perfluorochemicals are comprised of carbon and fluoride, are chemically inert, and are insoluble in water.

They were originally developed as hydraulic fluids and transformer coolants but are used as oxygen carriers because of their ability to dissolve oxygen and carbon dioxide. PFCs have a high oxygen solubility and are emulsified with a surfactant for intravenous administration. Unemulsified PFCs can dissolve 40–56 ml of oxygen per 100 ml of liquid while emulsified PFC dissolves 5–8 ml O_2/100 ml. The oxygen content of PFC has a linear dependence on $P\text{O}_2$ and in order to transport physiological quantities of oxygen a very high oxygen tension is needed. This factor limits the use of PFCs as oxygen carriers to situations where high oxygen tension can be maintained (e.g., surgery). PFCs have a short intravascular half-life, can activate the complement cascade, and have a slow elimination (Rentko, 1992; Wohl and Cotter, 1995).

Fluosal-DA is a 20% (by weight) emulsion previously licensed by the FDA for use in humans during coronary angioplasty surgery. PFC can also be potentially used during tumor radiation therapy, vascular surgery, extracorporeal organ perfusion, peritoneal lavage, and carbon monoxide poisoning (Rentko, 1992; Wohl and Cotter, 1995).

The half-life of perfluorochemicals depends upon the molecular size and structure of the agent. PFCs are expired in the lungs after phagocytosis by fixed macrophages, primarily in the liver and spleen (Wohl and Cotter, 1995).

Hemoglobin-Based Oxygen Carriers

Hemoglobin-based oxygen carriers (HBOCs) are useful for replacing red blood cells in anemic humans and animals. These products contain purified hemoglobin, removed from red blood cells and suspended in solution, and are especially useful when compatible red blood cells are not available. These solutions can pass through microcirculation more readily than red blood cells making them ideal for treating severe anemia or hypovolemia due to acute hemorrhage or poor blood flow distribution (Callan and Rentko, 2003; Lichtenberger, 2004).

HBOCs are developed from animal or human hemoglobin. Hemoglobin is an iron-containing protein composed of a tetramer of two α-chains and two β-chains with a heme molecule within the folds of each chain. When hemoglobin is administered, it breaks down into its constituent chains and has toxic effects (Awasthi, 2005). The dissociation is also associated with a short circulation half-life (Rentko, 1992). Small-sized, modified hemoglobin chains tend to extravasate, sequester nitric oxide, and cause vasoconstriction (Awasthi, 2005).

Cross-linking, glutaraldehyde polymerization, or polyethylene glycolation of hemoglobin prevents dissociation, improves the circulation half-life, and increases the size of the molecule to reduce extravasation (Rentko, 1992; Awasthi, 2005). The amount of cross-linking, polymerization, or polymer-linking also affects the viscosity of HBOC solutions. HBOCs cause an increase in plasma hemoglobin concentration and act as colloids to cause volume expansion (Wohl and Cotter, 1995; Awasthi, 2005).

Encapsulation is another technique used to stabilize hemoglobin. Hemoglobin can be encapsulated in nonantigenic phospholipids (liposomes), which prevents glomerular filtration and increases the half-life compared to free hemoglobin. Initial problems associated with encapsulated hemoglobin are related to reticuloendothelial stimulation, contamination with endotoxins, and rapid clearance (Rentko, 1992; Awasthi, 2005).

The only commercially available HBOC approved by the FDA for use in veterinary species is Oxyglobin® (OPK Biotech LLC, Cambridge, MA). Oxyglobin (hemoglobin glutamer-200 [bovine]), approved for use in the United States for treatment of anemia in dogs, is given as a single dose (10–30 ml/kg, 1.3–3.9 g/kg) administered at a rate no greater than 10 ml/kg/h (Hamilton et al., 2001; Callan and Rentko, 2003). This product is a purified and chemically modified bovine hemoglobin, which is stable at room temperature, has a shelf-life of 3 years, and has a similar oxygen-carrying capacity as endogenous hemoglobin (Senior, 1998; Callan and Rentko, 2003; Awasthi, 2005). The hemoglobin is polymerized to glutaraldehyde and is reconstituted in a modified lactated Ringer's solution (Hamilton et al., 2001; Lichtenberger, 2004). Oxyglobin does not need to be cross-matched and does not have the potential to transmit disease (Lichtenberger, 2004); therefore, it may be useful in emergencies when there is not time to cross-match or prepare blood products (Lanevschi and Wardrop, 2001).

The product has an osmolality of 300 mOsm/kg, lower viscosity than whole blood, hemoglobin concentration of 13 g/dl, colloidal oncotic pressure of 20–25 mm Hg, and a half-life of around 36 hours (30–40 hours) (Rentko, 1992; Senior, 1998; Callan and Rentko, 2003; Lichtenberger, 2004). It has a pH of 7.8 and expands the intravascular space by at least its own volume (Lichtenberger, 2004). Oxyglobin is not associated with renal toxicity, and there is no clinical evidence of allergic reaction with repeated doses (Rentko, 1992). Hamilton et al. (2001) demonstrated that multiple administrations of Oxyglobin (nine administrations over 50 weeks) in eight splenectomized dogs produced neither adverse physiological effects nor adverse pathological effects. Although most dogs produced Oxyglobin-specific IgG antibodies, no anaphylactic or anaphylactoid reactions were noted and the development of IgG antibodies did not affect the oxygen-carrying capacity (Senior, 1998; Hamilton et al., 2001).

The oxygen affinity of Oxyglobin is dependent on chloride ion concentration rather than the concentration of 2,3-diphosphoglycerate (2,3-DPG) (Lichtenberger, 2004). In humans and dogs the oxygen affinity of hemoglobin is determined by the interaction of hemoglobin and 2,3-DPG in red blood cells (Rentko, 1992). The levels of 2,3-DPG decrease in blood stored over 1 week, which leads to increased oxygen binding and decreased delivery of oxygen to the tissues. Compared to canine blood, Oxyglobin has a lower oxygen affinity enhancing oxygen delivery to tissues (Lichtenberger, 2004). One gram of hemoglobin from Oxyglobin can deliver the same amount of oxygen to tissues as 3–4 grams of red blood cell hemoglobin (Callan and Rentko, 2003).

Twenty-four hours after being opened, the product must be discarded due to the production of methemoglobin and to avoid bacterial contamination (Callan and Rentko, 2003; Lichtenberger, 2004). Oxyglobin is stored in its deoxygenated state and becomes oxygenated when it passes through the lungs (Senior, 1998). The primary effects of Oxyglobin last about 24 hours, and 90–95% percent of Oxyglobin is eliminated in 5–9 days (Senior, 1998; Callan and Rentko, 2003; Lichtenberger, 2004).

The volume of distribution, plasma clearance, and terminal elimination half-life of Oxyglobin are dose dependent, and administration of this product increases plasma hemoglobin concentrations. Medications should not be added to the bag, and fluids and drugs should not be administered through the same infusion set as Oxyglobin (Callan and Rentko, 2003).

Hemopure® (hemoglobin glutamer-250 [bovine]) (OPK Biotech LLC, Cambridge, MA) is a glutaraldehyde-polymerized bovine hemoglobin similar to Oxyglobin approved in South Africa for use in humans (Callan and Rentko, 2003; Awasthi, 2005). Hemopure can transport oxygen, needs no refrigeration, is compatible with all blood types, has a long shelf life, and has a minimized risk of disease transmission (Fitzpatrick et al., 2005). Fitzpatrick et al. (2005) demonstrated in laboratory studies that Hemopure reversed anaerobic metabolism in the treatment of hemorrhagic shock without end-organ damage. The decreased urine output noted in animals given Hemopure in laboratory studies has been attributed to the smaller volumes needed to resuscitate the animals and has not been associated with long-term decreased renal function (Fitzpatrick et al., 2005). Animal studies with Hemopure have demonstrated that it can carry oxygen more efficiently than red blood cells on a per-gram basis (Hamilton et al., 2001).

PolyHeme® (Northfield Laboratories, Evanston, IL) is a glutaraldehyde-polymerized human hemoglobin-based product (Awasthi, 2005).

Hemospan® (MP4 or MPEG-Hb) (Sangart, San Diego, CA) is a polyethylene glycolated hemoglobin (Awasthi, 2005; Björkholm et al., 2005). MP4 is very viscous, made from stroma-free human hemoglobin, has a high oxygen affinity, and is reported to not cause vasoconstriction (Awasthi, 2005; Björkholm et al., 2005). Based on in vitro and animal studies, the lack of vasoconstriction (i.e., avoidance of engaging autoregulatory vasoconstriction) is thought to be related to increased molecular size and oxygen affinity, which lead to a decreased diffusive oxygen transfer in the plasma space (Björkholm et al., 2005).

Cross-linking or polymerization stabilizes the structure of HBOCs so that there is almost no urinary excretion. Some modified hemoglobin may still be deposited in renal tubules. Other sites of deposition for HBOCs are lymph, liver, and spleen. HBOCs use fixed macrophages in their metabolism, which may impair macrophage phagocytic ability (Wohl and Cotter, 1995).

Adverse Effects

One of the shortcomings of early HBOCs was nephrotoxicity associated with contamination with red blood cell stromal elements. Current HBOCs are ultrapurified to prevent contamination (Callan and Rentko, 2003).

HBOCs should be used cautiously in euvolemic or hypervolemic patients to avoid volume overload related to the colloidal effects of HBOCs. Cats and small mammals appear to be more predisposed to pulmonary edema following volume overload (Lichtenberger, 2004). Circulatory overload is dependent on rate of infusion and is most marked in dogs receiving greater than 10 ml/kg/h and cats receiving more than 5 ml/kg/h (Wohl and Cotter, 1995; Callan and Rentko, 2003). Because HBOCs are colloids, they should be used cautiously in patients with preexisting cardiopulmonary disease (Callan and Rentko, 2003).

Ischemia–reperfusion injury is common to all HBOCs and occurs due to production of free radicals by hemoglobin and its oxidation products (Awasthi, 2005). HBOCs can provide oxygen and iron as electron donors leading to oxygen radical production and tissue damage (Wohl and Cotter, 1995).

Anaphylactic reactions are a possibility (though not documented in clinical or research cases) with repeated administration of heterologous HBOCs (Wohl and Cotter, 1995; Callan and Rentko, 2003; Awasthi, 2005). If hypersensitivity occurs, infusion of the HBOC should be discontinued, and appropriate resuscitation measures should be implemented (e.g., crystalloids, epinephrine).

Adverse effects of HBOCs in dogs, cats, and small mammals are discoloration of mucous membranes, sclera, and urine (Wohl and Cotter, 1995; Callan and Rentko, 2003; Lichtenberger, 2004). In bird species no discoloration of urine or mucous membranes is noted. Measurements of colorimetric lab tests are affected for 24–72 hours after Oxyglobin administration (Lichtenberger, 2004).

Hypertension can also be an adverse effect of hemoglobin solutions. HBOCs have a pressor effect that is attributed to sequestration, or scavenging, of nitric oxide following extravasation, but other mechanisms may be involved including endothelin release and sensitization of peripheral α-adrenergic receptors (Callan and Rentko, 2003; Fitzpatrick et al., 2005). Polymerization or polyethylene glycolation of hemoglobin decreases extravasation but does not completely eliminate its pressor effect (Awasthi, 2005; Fitzpatrick et al., 2005).

It is beyond the scope of this chapter to compare treatments for hemorrhagic shock, but much has been written on the topic, including therapy with blood substitutes. In a study comparing autologous/shed blood, hemoglobin-based oxygen carrier/Oxyglobin, crystalloid/saline, colloid/Hespan (6% hetastarch), and vasopressin in a canine hemorrhagic shock model (50–55% total blood loss with a mean arterial pressure of 45–50 mmHg as a clinical criterion), all resuscitation modalities except vasopressin restored microvascular and systemic function changes close to prehemorrhagic values. Autologous blood was the only treatment that restored oxygenation changes to prehemorrhagic levels (Cheung et al., 2007).

Special Topics

Horses

Horses present some special problems in acid–base management. In cases of severe diarrhea, shock, and intestinal obstruction, the horse seems predisposed to severe metabolic acidosis (Waterman, 1977). Electrolyte disorders common in horses with acute abdominal disease have been reviewed (Borer and Corley, 2006a,b). Respiratory acidosis is a very common sequel to closed-circuit inhalation anesthesia in the horse. An abnormally low concentration of Na^+ is a common problem in dehydrated horses. Severe hypokalemia, with blood K^+ values less than 2.5–3 mEq/l, may require treatment with solutions high in K^+. Dangerous hyperkalemia, with blood levels greater than approximately 7 mEq/l, may be associated with acidosis in foals. Prompt correction of the acidosis will usually correct the hyperkalemia.

Cattle

Ruminants also present special fluid and electrolyte management problems. When a diagnosis of abomasal disease is coupled with an obvious fluid balance disorder, hypochloremia, hypokalemia, and alkalosis are usually present. These should be confirmed by appropriate laboratory tests. Grain overloading will result in severe dehydration and metabolic acidosis. Calf diarrhea also results in severe dehydration and metabolic acidosis, with dangerous hyperkalemia in some cases. If hyperkalemia exists, one must guard against administration of even more K^+. When dealing with herbivores, it is important to remember that normal feed contains high levels of K^+. When these animals are anorexic, they frequently become K^+ depleted. The best way to replace K^+ deficits is by consumption of hay or grass, but K^+ must be added parenterally when the situation dictates. A wide variety of electrolyte mixtures containing K^+ are available for oral administration.

Intravenous administration of 5% dextrose alone or with isotonic sodium bicarbonate to hypernatremic, diarrheic calves has been preliminarily observed to provide benefit (Abutarbush and Petrie, 2007). Despite the fact that the average reduction rate of serum sodium concentration in these calves ($n = 5$) was about four times that recommended, no complications were reported in this small cohort. Further studies are needed to determine how this approach compares with other therapies.

Anesthetic and Surgical Effects

General anesthesia may exert several effects on water, electrolyte, and acid–base balance. Almost all general anesthetics induce some degree of Ca^{++} channel blockade, resulting in some degree of vasodilation and myocardial depression. The end effect can be a reduction in cardiac output and/or alterations in organ blood flow. Arterial pressures are frequently lowered in a dose-dependent manner, and GFR may be affected. The commonly used inhalation anesthetic agents (halothane, enflurane, and isoflurane) all cause direct systemic vasodilation. Narcotics and some muscle relaxants also can cause vasodilation. As a result of the vasodilation, fluid requirements may be increased during the course of the surgical procedure to maintain adequate blood pressure and cardiac output. After recovery from the general anesthetic, when vascular tone is normalized, the patient may be volume overloaded and hypertensive. Fluid loss may also increase during general anesthesia as a result of tracheal intubation and/or artificial ventilation. Normal mechanisms for the humidification of inspired air are bypassed, and the cold, dry gases from the anesthesia machine can cause a considerable amount of fluid loss. Open body cavities allow for evaporative losses. Third spacing may occur with extravasation of fluid from the vascular to the extravascular, extracellular spaces. If extravasated fluid is replaced to maintain adequate circulatory volumes, the patient with inadequate cardiac reserve or poor renal function may suffer fluid overload and congestive heart failure when postoperative redistribution of the fluid back into the circulation occurs (Gold, 1992).

Surgical injury can result in significant reductions in serum albumin, total proteins, and total lymphocyte counts. These decreases are typically greater following abdominal surgery. The decreases have been found to be primarily caused by the volume of IV fluids frequently required for resuscitation and to compensate for blood loss.

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24

Diuretics and Renal Pharmacology

Butch Kukanich and Deborah T. Kochevar

This chapter presents the physiological basis for fluid and electrolyte balance, including discussion of selected renal mechanisms for regulation of water, sodium, chloride, potassium, hydrogen, and bicarbonate. In this chapter these concepts will be extended in order to understand the mechanism of action, therapeutic uses, and side effects of diuretic agents and renal pharmacology. Diuretic agents are used to mobilize tissue fluid, most often in the treatment of edema of cardiac, renal, or hepatic origin. The history of diuretics dates back to consumption by Paleolithic humans of caffeine-containing plants. Besides xanthine derivatives such as caffeine, osmotic diuretics were clinically important prior to the 20th century. The use of mercurial diuretics, now therapeutically obsolete, began in the early 1900s and was followed by introduction of the first modern diuretic, acetazolamide, in the mid-1950s. By the late 1950s and early 1960s the formulary of modern diuretics included chlorothiazide, furosemide, and potassium-sparing diuretics (Morrison, 1997). These drugs and their relatives constitute the mainstays of diuretic treatment. Finally, the renin–angiotensin–aldosterone system will be discussed, which can be activated by different mechanisms resulting in local and systemic effects including vascular and renal effects.

Renal Physiology

Nephron Function

Knowledge of renal anatomy and physiology is essential to understanding the mechanism of action of diuretic drugs. Although a thorough review of these topics is beyond the scope of this text, a brief overview of nephron function is provided. The basic functional unit of the kidney is the nephron, which consists of a filtering apparatus, the glomerulus, connected to an extended tubular structure that reabsorbs and conditions the glomerular ultrafiltrate to produce urine. Each kidney is composed of thousands of nephron units. Figures 24.1 and 24.2 are drawings of single nephron units, indicating the broad subdivisions of nephron segments and the sites of action of diuretic agents. This diagram provides the simplest nomenclature for nephron segments. As knowledge of the function and epithelial morphology of each segment has increased, the tubular portion of the nephron has been subdivided into approximately 14 shorter segments referred to by a standardized nomenclature (Kriz and Kaissling, 1992).

Blood flow through the kidney goes from the renal artery into smaller arteries until it reaches the afferent arteriole (Figure 24.1). The afferent arteriole becomes the glomerular capillaries (where glomerular filtration occurs) then the efferent arterioles. The efferent arterioles carry blood into the peritubular capillaries, which surround the renal tubules and is where the majority of the glomerular filtrate (water, electrolytes, glucose, etc.) is reabsorbed.

Formation of urine starts in the glomerulus, where a portion of plasma water is filtered through fenestrated glomerular capillary endothelial cells, a basement membrane, and, finally, filtration slit diaphragms formed by the visceral epithelial cells that cover the basement membrane on its urinary space side. The filtrate collects in Bowman's space, a double-walled invagination that surrounds the glomerular capillaries. From Bowman's capsule the filtered fluid passes into the proximal tubule and begins its passage through the renal tubular system. Small solutes (e.g., sodium, chloride, glucose) are actively filtered with plasma water while larger elements, such as protein, blood cells, and macromolecules, are retained within the glomerular capillaries. The rate of filtration in each nephron is a function of hydrostatic pressure in the glomerular capillaries, hydrostatic pressure from the ultrafiltrate in Bowman's space, mean colloid osmotic pressure in the glomerular capillaries, colloid osmotic pressure of the ultrafiltrate in Bowman's space, and the properties of the filtering membrane. Hydrostatic pressures are the pressures of the fluid against the surface membrane; in the glomerular capillaries, hydrostatic pressure is determined by capillary blood pressure and in

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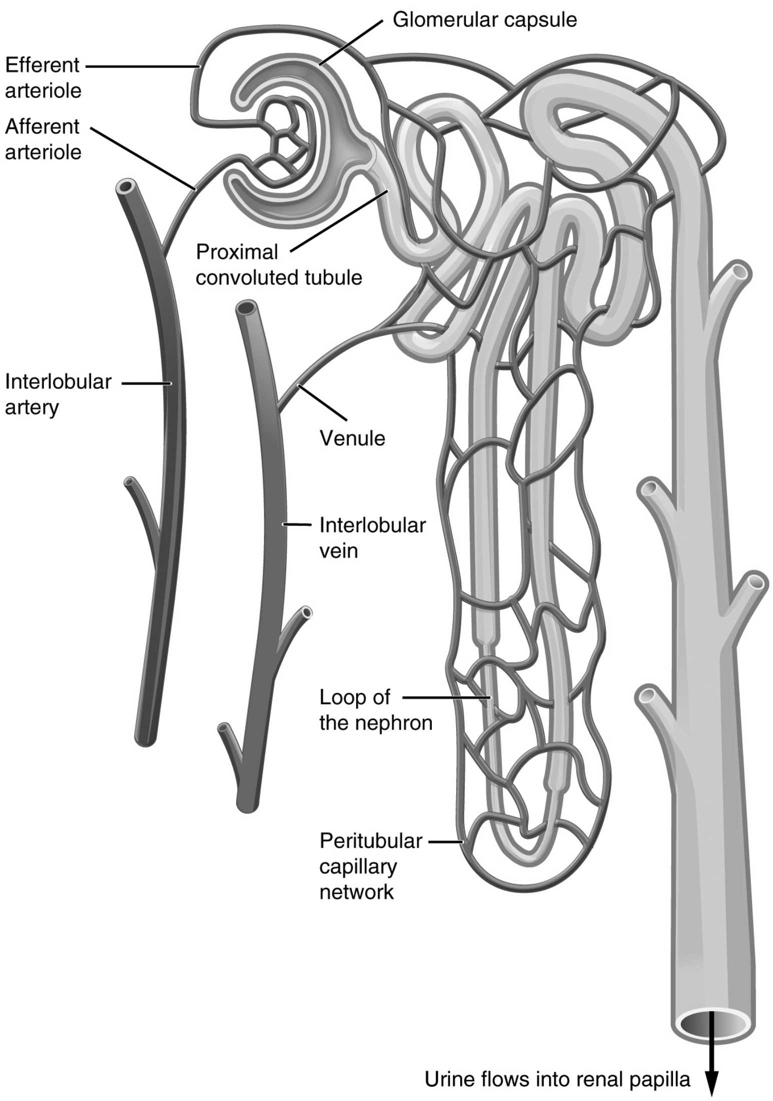

Figure 24.1 Anatomy of the nephron and associated structure. Source: http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.30. Used under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/.

the Bowman's space by the pressure of the ultrafiltrate. The primary constituent of the glomerular capillary colloid osmotic pressure is albumin in the plasma and there is little colloidal osmotic pressure in normal urine produced by healthy glomeruli. The colloid osmotic pressure in the plasma is higher than the glomerular filtrate, resulting in an opposing force to filtration. The net filtration pressure (Figure 24.3) is determined by the following relationship:

Net filtration pressure = (blood hydrostatic pressure)
– (blood colloid osmotic pressure)
– (ultrafiltrate hydrostatic pressure)

Changes in glomerular capillary hydrostatic pressure (e.g., hyper- or hypotension), blood colloid osmotic pressure (e.g., hypoalbuminemia), and ultrafiltrate hydrostatic pressure (e.g., albuminuria) can have profound effects on glomerular filtration.

Ultrafiltrate from the glomerulus enters the proximal tubule from Bowman's capsule. By the time urine exits the distal tubule and collecting duct, better than 99% of ultrafiltrate volume will be reabsorbed. Figure 24.4 summarizes the characteristics of reabsorption in broad sections of the renal tubules.

The proximal convoluted tubule (PCT) reabsorbs the majority of sodium (~60% that enters the nephron) by various transporters and water passively follows and is eventually absorbed into the peritubular capillaries by osmosis. Nearly 100% of the glucose, amino acids, and other substances such as vitamins are also reabsorbed in the PCT by various transporters. Animals with hyperglycemia (e.g., diabetes mellitus) may have large amounts of glucose in the nephron, which exceeds the transport capacity of the active transporters to reabsorb from the nephron and glycosuria can occur. Approximately 90% of bicarbonate is reabsorbed in the PCT through the activity of the enzyme carbonic anhydrase (CA) (Figure 24.5).

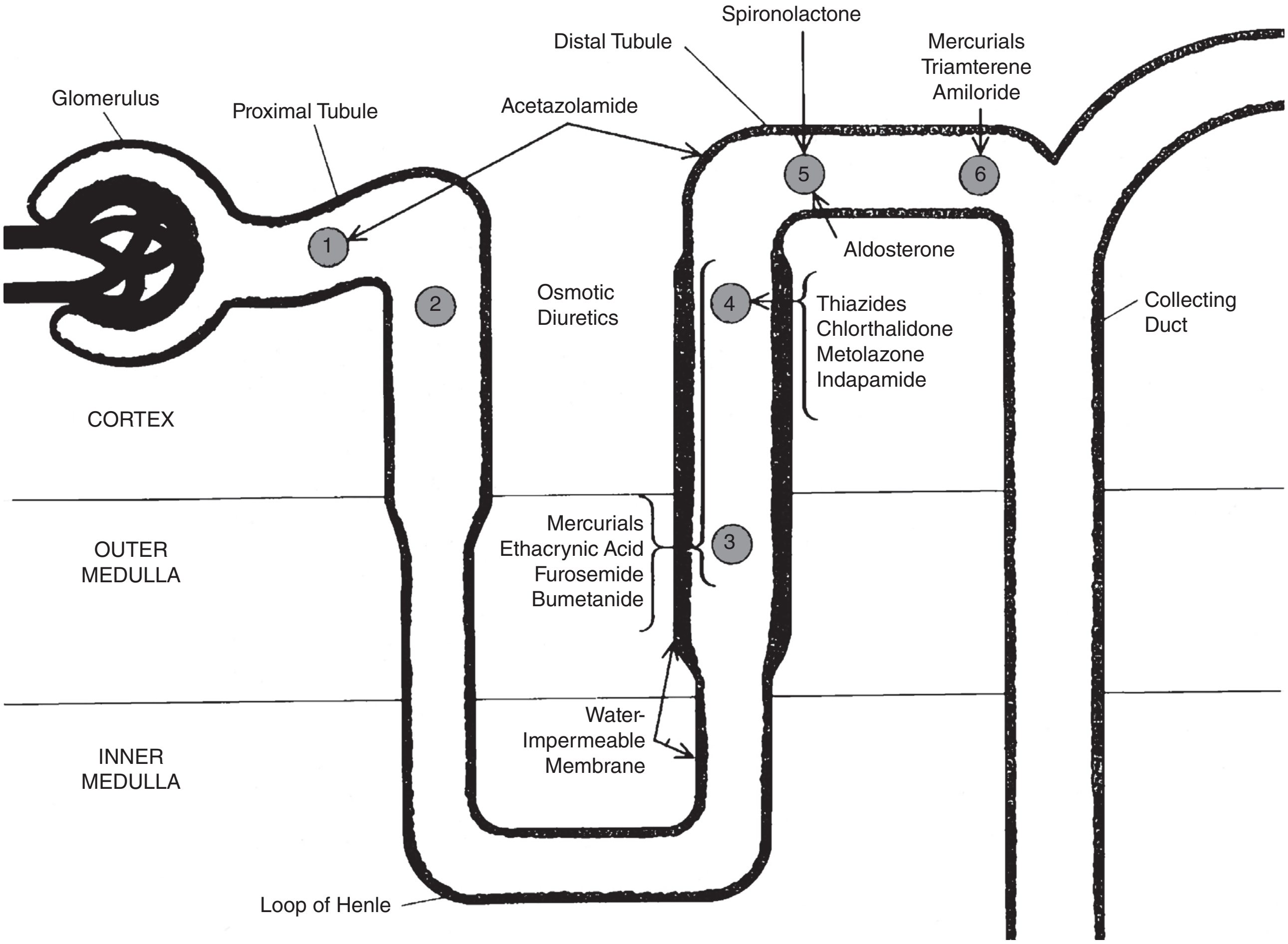

Figure 24.2 Locations within the nephron where diuretic agents exert their effects.

In the nephron bicarbonate combines with a hydrogen ion to produce carbonic acid. Carbonic acid is converted to water and carbon dioxide by CA and carbon dioxide freely diffuses into the PCT cell. Carbon dioxide in the PCT cell then combines with water to form carbonic acid, catalyzed by CA, and the carbonic acid dissociates into bicarbonate and a hydrogen ion effectively transporting bicarbonate from the nephron into the PCT cell. Bicarbonate is then cotransported with sodium to the interstitial fluid where it can diffuse into the peritubular capillaries. Carbonic anhydrase inhibitors (e.g., acetazolamide, methazolamide) exert their primary effects in the PCT, but also have effects on the distal convoluted tubule (DCT).

The Loop of Henle consists of a thick descending loop, thin descending loop, thin ascending loop, and thick ascending loop. Water pores (aquaporins) are present in the thin descending loop that allow water to osmotically move out of the nephron into the interstitial space, which is hyperosmolar (up to four times osmolarity as the original nephron contents). Reabsorption of sodium, potassium, and chloride from the nephron into the renal tubule cell by the sodium-potassium-2-chloride ($Na^+/K^+/2Cl^-$) cotransporter occurs in the thick ascending loop. Sodium is then transported from the cell to the interstitial fluid by the sodium/potassium ATPase and chloride follows sodium through chloride channels to the interstitial fluid (Figure 24.6). The thick ascending loop is impermeable to water. The high osmolarity of the interstitial fluid is thus maintained by the net movement of sodium and chloride from the nephron to the interstitial fluid, which transports a large portion of the sodium and chloride (~35% of the original ultrafiltrate) (Figure 24.7).

The thick ascending limb of the loop of Henle is of particular importance since this is the site of action of the most effective diuretic drugs (i.e., loop diuretics, furosemide). Approximately 25% of filtered solutes are reabsorbed in the loop of Henle, and most of this reabsorption occurs in the thick ascending limb. The thick ascending loop connects with the distal convoluted tubule to make a critical contact with the afferent arteriole through a cluster of specialized epithelial cells, referred to as the macula densa, which monitors the sodium and tubular flow rates and is discussed in more

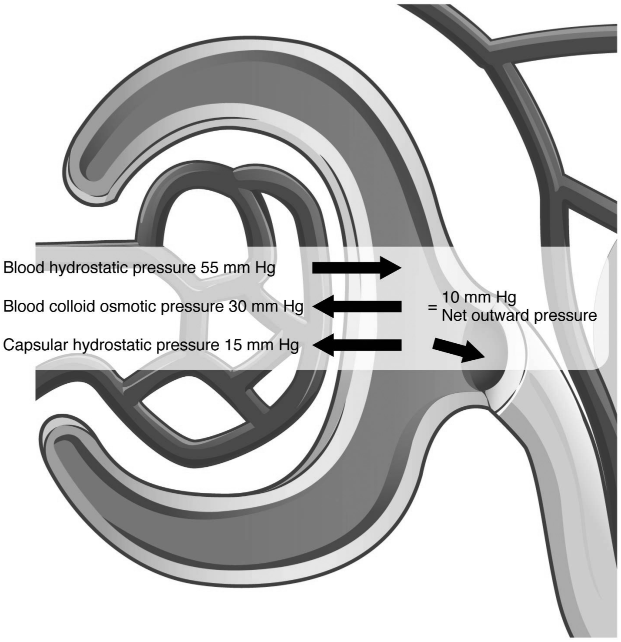
Blood hydrostatic pressure 55 mm Hg
Blood colloid osmotic pressure 30 mm Hg
Capsular hydrostatic pressure 15 mm Hg
= 10 mm Hg
Net outward pressure

Figure 24.3 The glomerular net filtration pressure. Source: http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.30. Used under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/.

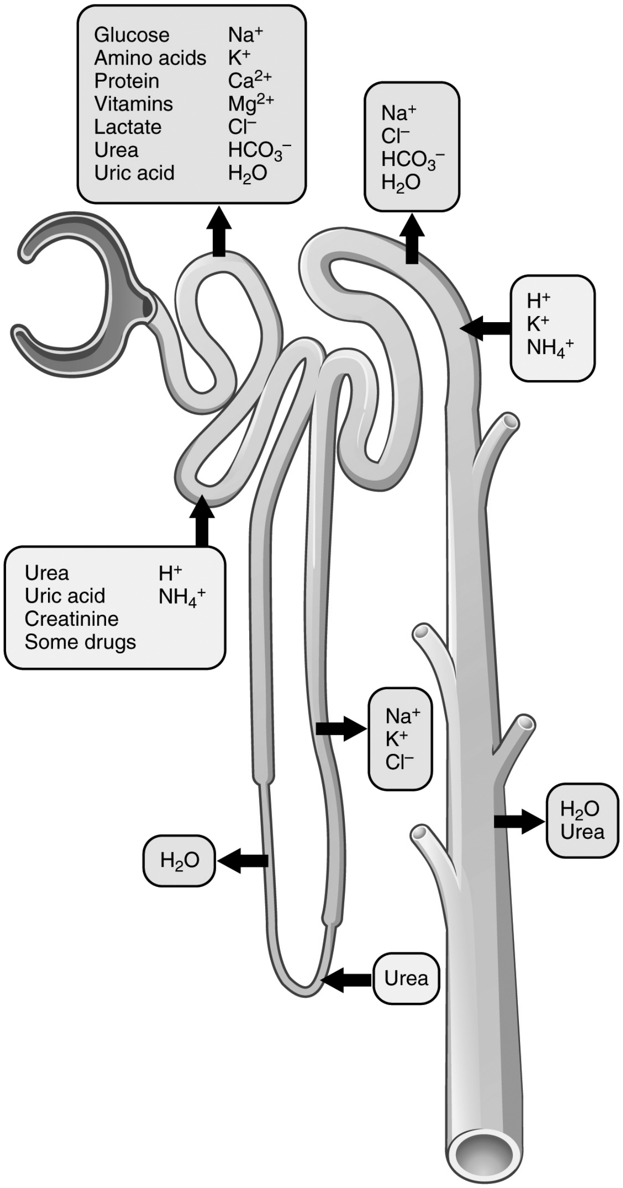

Figure 24.4 Nephron ion and water movement. Source: http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.30. Used under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/.

detail in Section Renin–Angiotensin–Aldosterone System (Figure 24.8). Water, sodium, chloride, bicarbonate, and calcium are reabsorbed in the DCT. Sodium/chloride symporters transport sodium and chloride from the nephron into the DCT cells and are sensitive to the thiazide diuretics (Figure 24.9). Aldosterone enhances sodium and water reabsorption in the DCT and collecting duct by increasing the amount of sodium/potassium ATPase transporters moving sodium out of the nephron and chloride follows along with water. Spironolactone, an aldosterone antagonist, is a diuretic that exerts its effects in the DCT and the collecting duct. Parathyroid hormone (PTH) receptors on the DCT when bound by PTH insert calcium channels on the luminal surface to enhance recovery of calcium from the tubules.

The collecting duct produces the final effects on urine volume based on plasma osmolarity. More water is reabsorbed if the plasma osmolarity is high (e.g., dehydration) and more water is lost if the plasma osmolarity is low (e.g., overhydration). Two main cell types are present in the collecting duct, the principal cells and intercalated cells. The principal cells possess channels for the recovery or loss of sodium and potassium and the intercalated cells secrete or absorb acid and bicarbonate. Intercalated cells are important factors in regulating urine (and plasma) pH through absorption of bicarbonate and excretion of hydrogen ions. Antidiuretic hormone (ADH, arginine vasopressin, or specifically in swine lysine vasopressin) is released from the posterior pituitary gland when plasma osmolarity increases with an effect of increasing water reabsorption in the collecting duct through aquaporins (water channels). Antidiuretic hormone stimulates aquaporin channels to be inserted on the apical side (tubular side) of the principal cells, resulting in water movement from the nephron into the principal cells due to an osmotic gradient. Different aquaporin channels on the basolateral cell membrane allow water movement from the cell into the interstitial space by osmotic movement. The water than diffuses into the peritubular capillaries and reenters the circulation. Alcohol (ethanol) consumption decreases ADH resulting in a diuretic effect,

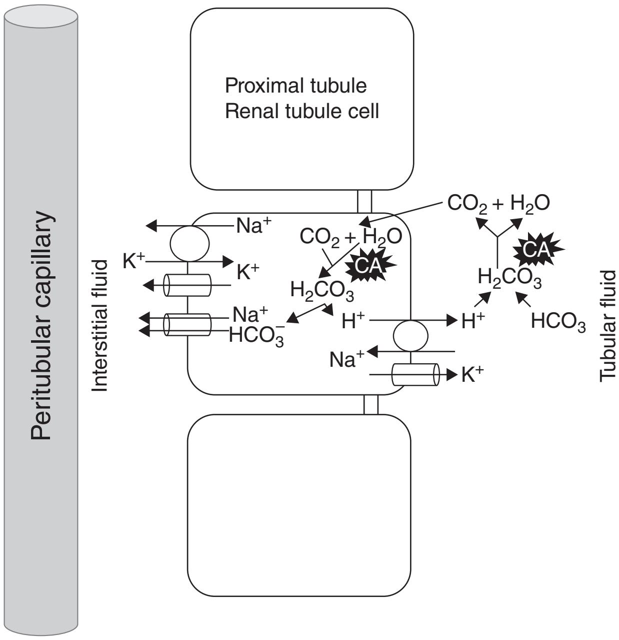

Figure 24.5 Reabsorption of bicarbonate from the proximal convoluted tubule, the primary location of carbonic anhydrase inhibitor effects.

which is observed in animals treated for ethylene glycol toxicity. Aldosterone also produces an effect on the collecting duct, resulting in increased sodium reabsorption from the collecting duct by sodium/potassium channels and sodium/potassium ATPases. Water passively follows sodium, resulting in a net increased reabsorption of sodium and water and net excretion of potassium. Spironolactone administration results in inhibition of aldosterone leading to loss of sodium and water and retention of potassium from the collecting duct and is often referred as a “potassium-sparing” diuretic.

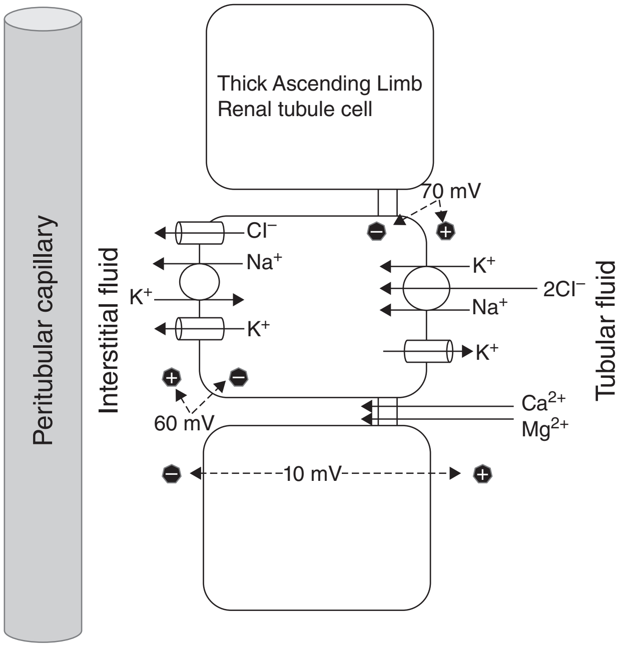

Figure 24.6 Ion movement in the thick ascending loop, the primary location of furosemide effects.

Factors Regulating Renal Function

Renin–Angiotensin–Aldosterone System

The macula densa is located at the junction of the thick ascending limb and distal convoluted tubule and sits between the afferent and efferent arterioles. Together with the juxtaglomerular cells, which produce renin, these components form the juxtaglomerular apparatus (Figure 24.8). Increased reabsorption of Na and Cl detected by the macular densa results in inhibition of renin release into the efferent arteriole by the juxtaglomerular cells through activation of adenosine (A_1) receptors. Conversely, decreased reabsorption of Na and Cl detected by the macular densa stimulates renin release into the efferent arterial through prostaglandins (PGE_2, PGI_2). Renin release is also enhanced when low blood pressure is detected by intrarenal baroreceptors, triggering the release of prostaglandins (PGE_2, PGI_2). Renin release can also be stimulated by an extrarenal mechanism, sympathetic nerve stimulation of β_1 receptors on the juxtaglomerular cells.

Renin release from the juxtaglomerular cells results in a cascade of events known as the renin–angiotensin–aldosterone system (RAAS) (Figure 24.10). Renin converts angiotensinogen, which is released from the liver, to angiotensin I (ATI). Angiotensin I is converted to angiotensin II (ATII) by angiotensin converting enzymes (ACE) located in the lungs. Angiotensin II is a vasoconstrictor, which binds to AT receptors in the vasculature resulting in increased vascular tone and increased blood pressure. Angiotensin II also stimulates the release of aldosterone from the adrenal cortex, which enhances sodium and subsequently water reabsorption in the distal convoluted tubule and collecting ducts, increasing circulating blood volume and blood pressure.

ATII increases vascular tone by multiple mechanisms. ATII produces direct vasoconstriction through AT receptors, resulting in the rapid pressor response. Peripheral sympathetic neurotransmission is enhanced by ATII, resulting in increased release of norepinephrine from nerve terminals. Centrally mediated enhanced sympathetic outflow is also stimulated by ATII. ATII also stimulates catecholamine release from the adrenal medulla.

ATII has affects on renal function. ATII stimulates Na^+/K^+ exchange in the PCT, which increases the reabsorption of Na^+, Cl^-, and $HCO3^+$ (Figure 24.5). The expression of the Na^+/glucose transporter in the PCT

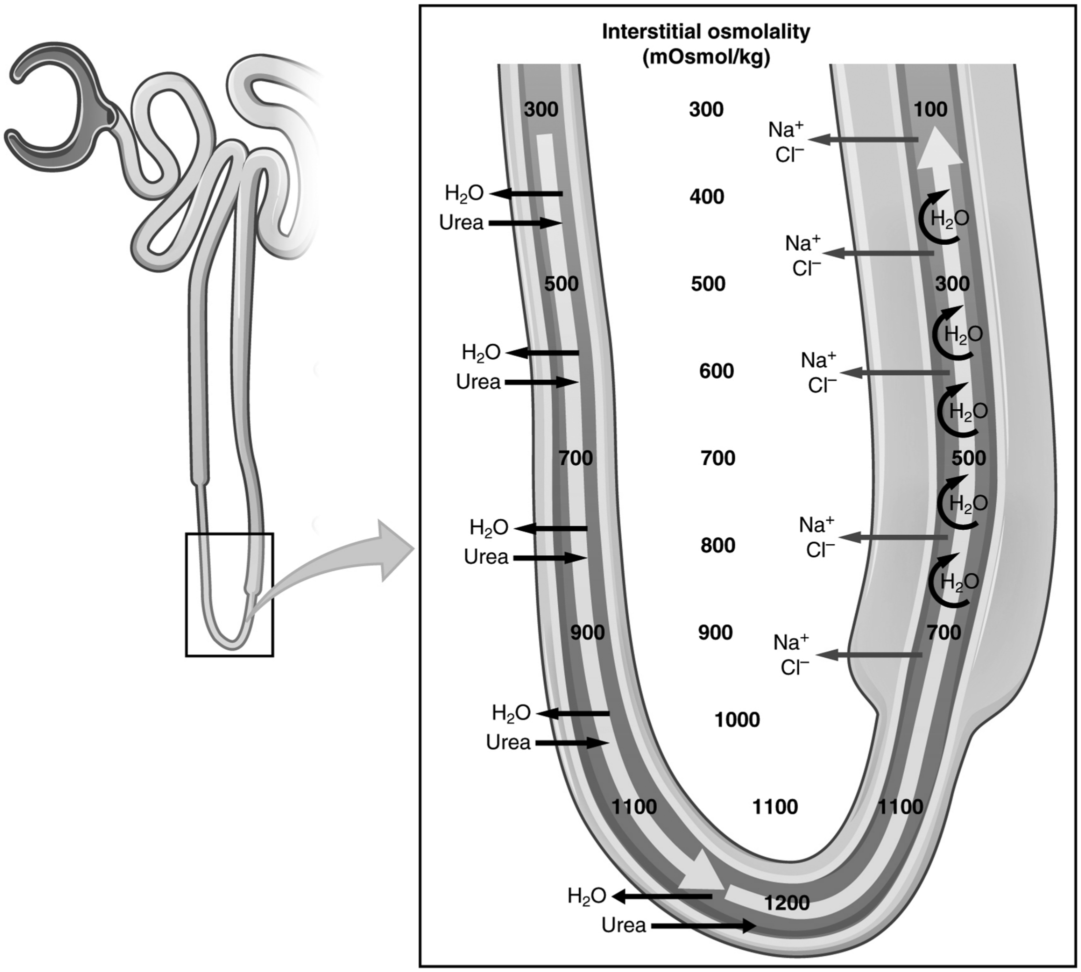

Figure 24.7 The loop of Henle countercurrent multiplier system and osmolality of the interstitial fluid and tubular fluid. Source: http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.30. Used under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/.

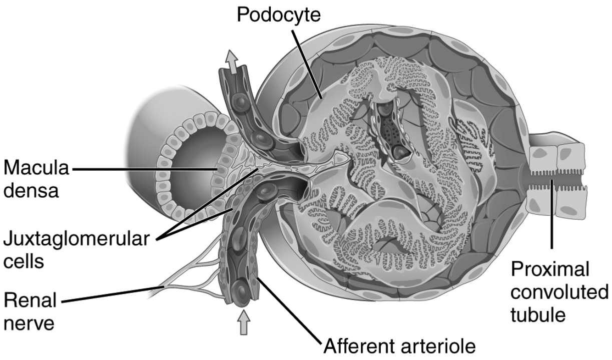

Figure 24.8 Anatomy of the glomerulus, macula densa, juxtaglomerular cells and renal vasculature. Source: http://cnx.org/contents/14fb4ad7-39a1-4eee-ab6e-3ef2482e3e22@7.30. Used under CC BY 4.0 https://creativecommons.org/licenses/by/4.0/.

is increased with low concentrations of ATII. The activity of $Na^+/K^+/2Cl^-$ transporter in the thick ascending limb is also enhanced. ATII stimulates the release of aldosterone, which acts at the DCT and collecting ducts to enhance retention of Na^+ and water while increasing elimination of K^+. The vasoconstriction induced by ATII in most cases decreases GFR by affecting the afferent arteriole greater than the efferent arterial. However, during renal hypotension ATII affects the efferent arterial to a greater extent than the afferent, resulting in increased GFR. Administration of ACE inhibitors during renal hypotension increases the risk of acute renal failure.

The RAAS production of ATII is also associated with altered cardiovascular structures. Increased cardiac afterload, due to increased vascular tone, and increased preload, due to aldosterone-mediated sodium and water retention, contribute to cardiac remodeling and hypertrophy. Direct effects of ATII on cardiac myocytes, vascular smooth muscle, and fibroblasts also result

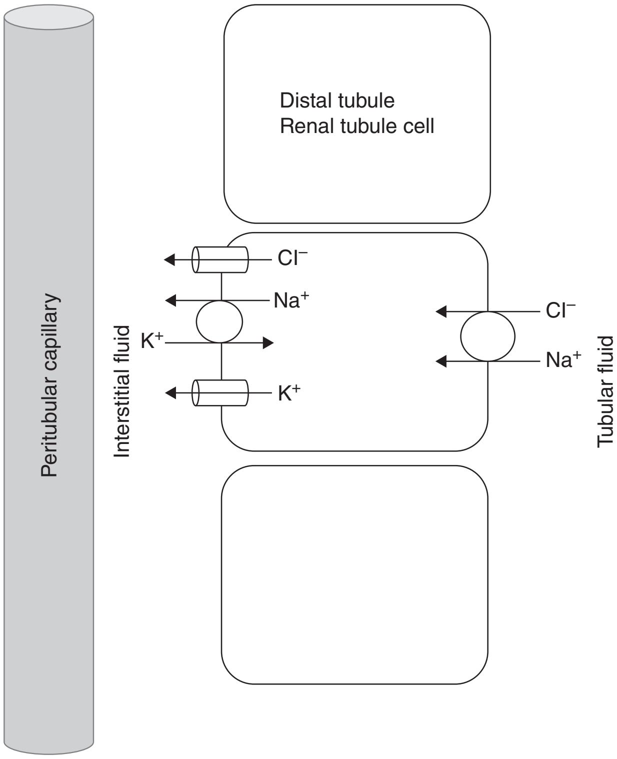

Figure 24.9 Ion movement in the distal convoluted tubule, the primary location of thiazide diuretic effects.

in cardiac hypertrophy and remodeling and vascular remodeling resulting in increased vascular wall thickness and decreased vascular compliance.

Renin release is modulated by feedback inhibition. Angiotensin II stimulates AT receptors on the juxtaglomerular cells decreasing the release of renin and is known as the short negative feedback loop. Increases in blood pressure due to ATII vasoconstriction results in decreased sympathetic tone and subsequently β_1 receptor stimulation. Increases in afferent arterial pressures decrease renin release and is termed the long loop negative feedback.

The RAAS is classically described as an endocrine system response as described above in this section, but locally active and alternative pathways for angiotensin synthesis are present. ACE is present throughout the vascular system in endothelial cells and circulating renin can be stored in the endothelial cells, which can subsequently locally activate the RAAS (Mompeón et al., 2015). Addition tissues (brain, vasculature, heart, adrenal glands, etc.) can produce renin, AT, or ACE that affect local tissue function and structure (Bader and Ganten, 2008). There are also other enzymes that can contribute to the metabolism of angiotensinogen to ATI and ATII. Chymases are enzymes implicated in production of ATII from angiotensin in tissues that can be refractory to ACE inhibitor therapy. There appears to be species-specific

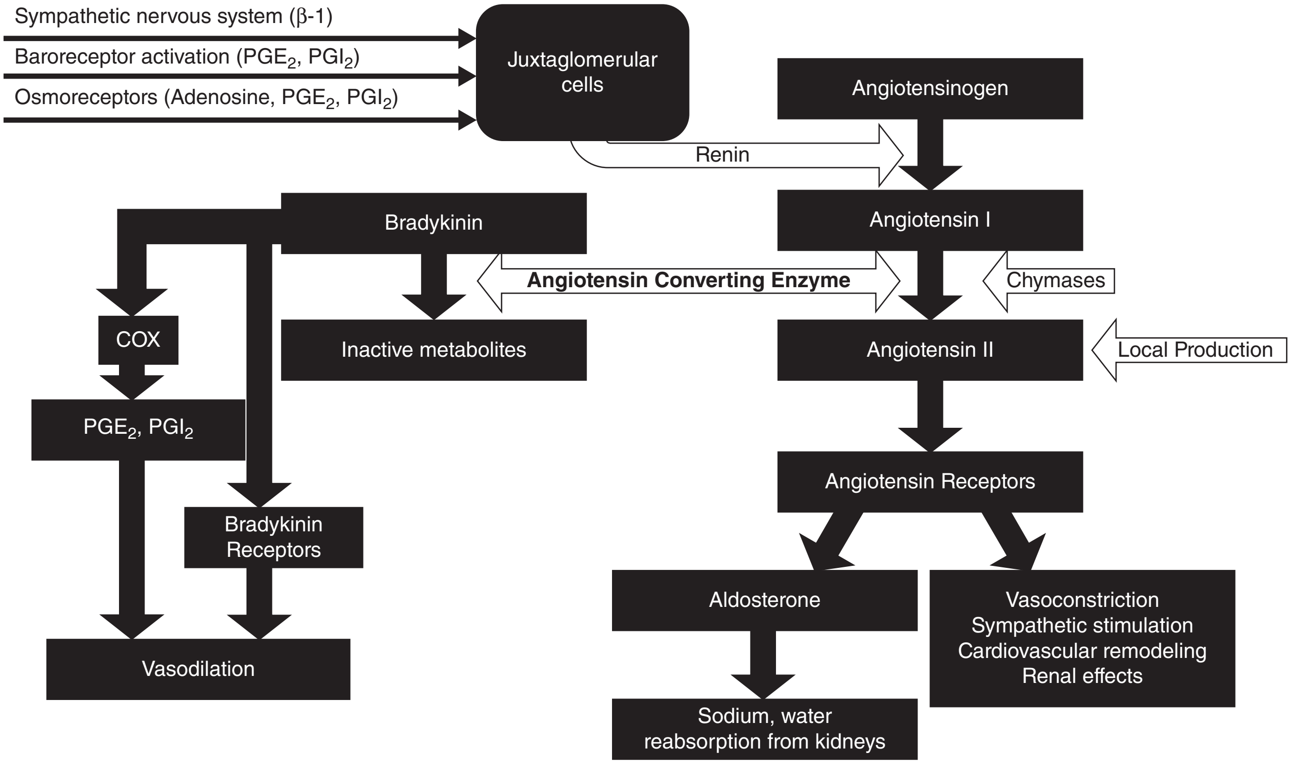

Figure 24.10 Schematic of the renin–angiotensin–aldosterone system.

differences and the overall contribution to ATII formation appears much less than ACE (Aramaki et al., 2003; Campbell, 2012).

Sympathetic Nervous System

The kidneys are innervated by the sympathetic nervous system. Adrenergic α_1-receptor activation results in vasoconstriction of the afferent arterial resulting in decreased blood flow, decreased hydrostatic pressure in the glomerulus, and decreased glomerular filtration rate. Sympathetic β_1 receptors are also located on the macula densa, which stimulate renin release and activation of the RAAS.

Antidiuretic Hormone

Osmoreceptors are specialized cells in the hypothalamus that are sensitive to changes in blood osmolarity (primarily influenced by sodium ion concentration). If blood osmolarity increases, typically due to sodium ingestion or water deprivation), ADH is released from the posterior pituitary with its primary effects on the collecting duct cells to increase water reabsorption from the tubular fluid by inserting aquaporins on the apical (lumen) side of the cells. ADH release is controlled by a negative feedback loop in which low blood osmolarity detected by the osmoreceptors inhibits the release of ADH. Diabetes insipidus is a disease characterized by insufficient production of ADH, resulting in blood hyperosmolarity. Ethanol, which is used in the management of ethylene glycol toxicity, inhibits the release of ADH, which can cause diuresis. ADH is also known as vasopressin as high concentrations result in vasoconstriction.

Cyclooxygenase

Cylcooxygenase (COX) exists as at least two distinct enzymes (COX1, COX2) and is described in detail in Chapter 20. Both COX isoforms are constitutively expressed in the kidneys, but COX2 expression can be induced to produce substantial local effects within the kidneys during periods of hypotension or decreased blood flow. Eicosanoids, products of COX, can exert prominent roles in renal physiology during certain conditions including hypotension (cardiac disease, vasodilation), hypovolemia (dehydration, hemorrhage), and hyponatremia. COXs produce a variety of eicosanoids, including prostaglandin E_2 (PGE_2), prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), prostacyclin (PGI_2), and thromboxane ($TBXA_2$) with resultant effects in the kidney. The eicosanoids can produce afferent arteriole vasodilation (PGE_2, PGI_2) locally antagonizing the effects of systemic vasoconstriction such as sympathetic stimulation, ATII, and vasopressin. Eicosanoids enhance renin release (PGE_2, PGI_2), but renin release is not solely dependent on prostaglandins. Eicosanoids enhance elimination of sodium and water (PGE_2, PGI_2, $PGF_{2\alpha}$) through inhibition of Na^+/K^+ ATPase activity and aquaporin activity. Prostaglandins (PGE_2, PGI2) also produce local vasodilation to maintain medullary blood flow during states of systemic vasoconstriction. Therefore it is not surprising that COX inhibitors can have profound renal effects, with renal adverse effects of nonsteroidal antiinflammatory drugs being the second most common organ system affected with adverse effects.

Renal Epithelial Transport

The presence of anion and cation transporters is essential for most highly protein-bound diuretic drugs to gain access to their site of action, the lumen of the renal tubule. Loop and thiazide diuretics and carbonic anhydrase inhibitors are secreted through the organic acid pathway, and amiloride and triamterene via an organic base transporter (Brater, 1998). Renal insufficiency accompanied by reduced creatinine clearance decreases delivery of diuretic drugs to their secretory site and hence to their site of action. Accumulation of endogenous organic acids during chronic renal failure may result in competition with diuretics for transport at proximal tubule secretion sites (Brater, 1993). Other transporters, including the p-glycoprotein efflux pump, breast cancer resistance protein (BRCP), multidrug and toxin extrusion proteins (MATE1, MATE2-K), and multidrug resistance proteins (MRP2, MRP4), are located in the proximal tubule that contribute to transport of drug into the urine functioning as a mechanism of drug clearance (2013).

Principles of Diuretic Use

Overview

The current therapeutic goal of diuretic use is increased excretion of sodium followed by water. The degree of sodium loss in the urine (referred to as natriuresis or, in combination with chloride, saluresis) varies with the mechanism of action of the drug. All except osmotic diuretics inhibit specific enzymes, transport proteins, hormone receptors, or ion channels that function, directly or indirectly, in renal tubular sodium reabsorption. Although saluresis is the primary clinical goal, diuretics also alter elimination of other ions to varying degrees (e.g., K^+, H^+, Ca^{2+}, Mg^{2+}, Cl^-, HCO_3^-, phosphates) and may affect renal hemodynamics. Diuretic-induced depletion of circulating blood volume may lead to adverse effects such as electrolyte imbalances and dehydration if therapy is not well monitored. Older animals and those with cardiac or renal disease are also at increased risk for adverse effects if diuretic-induced hypovolemia goes untreated. Because these groups are also the primary target groups for diuretic use, rational

Table 24.1 The effect of diuretics on water and electrolyte elimination

	H_20	Na^+	K^+	Cl^-	Mg^{2+}	Ca^{2+}	HCO_3^-	H_2PO_4	H^+
Carbonic anhydrase inhibitors	+	+	++	0–+	+/–	0	++	++	–
Osmotic diuretics	++	++	+	+	++	+	+	+	?
Loop diuretics ($Na^+K^+2Cl^-$ symport)	++	++	++	++	++	++	0–+	0–+	+
Thiazide diuretics (Na^+/Cl^- symport)	+	+	++	+	+	-(chronic)	0–+	0–+	+
K^+-sparing (Na^+ channel)	+	+	–	+	–	–	+	0	–
Aldosterone antagonists	+	+	–	+	0	?	+	?	–

++, large increase in elimination; +, increase in elimination; 0, little to no change in elimination; – decreased elimination; +/– variable effect; ? effect unknown.

use of diuretic drugs is essential. Table 24.1 summarizes selected features of diuretic drugs most commonly used in veterinary medicine.

Edema Formation

The most common indication for diuretic use is removal of tissue edema. Understanding the physiological principles underlying edema formation depends upon an understanding of net capillary filtration. Similar to net filtration pressure in the glomeruli, net capillary filtration, or fluid flux out of a capillary, is dependent upon the plasma colloidal osmotic pressure retaining fluid, and hydrostatic pressure in the arterial capillary forcing fluid out of the capillary through fenestrations and pores. Since the arterial capillary hydrostatic pressure is greater than the plasma colloidal osmotic pressure in most tissues, a small amount of fluid moves from the vasculature into the interstitial space. However, on the venous capillary side, plasma osmotic pressure is greater than the venous hydrostatic pressure and fluid is reabsorbed from the interstitial space into the venous capillary in most tissues, resulting in little net fluid loss into the interstitial space. The lymphatics contribute to fluid absorption from the interstitial space, ensuring no accumulation of fluid in the interstitial space in healthy tissues.

If the fluid movement out of the capillaries exceeds absorption of fluid by venous capillaries and lymphatics, edema results. There can be numerous causes of edema including: lymphatic obstruction (i.e., neoplasia), decreased plasma osmotic pressure (i.e., hypoalbuminemia), and increased venous hydrostatic pressure (i.e., decreased cardiac contractility, excessive intravascular fluid volume). Additionally, the loss of capillary integrity (i.e., inflammation, neurogenic) can result in tissue edema and noncardiogenic pulmonary edema due to head trauma, seizures, and electrocution that occur as a result of poorly understood mechanisms. Therefore the cause of the edema should be identified prior to any therapy, including diuretics.

Congestive heart failure (CHF) is a complex pathophysiological process that begins with decreased cardiac output. The decreased cardiac output and decreased renal blood flow leads to activation of the renin–angiotensin–aldosterone system followed by renal retention of salt and water. High baroreceptor activity causes increased peripheral vascular resistance and increased ADH, which lead to further salt and water retention by the kidneys. Increased central venous pressure caused by increased left ventricular end-diastolic pressures cause increased capillary hydrostatic pressure. All of these factors lead to greater fluid flux out of vessels, resulting in edema related to cardiac disease.

Inhibitors of Carbonic Anhydrase

Chemistry/Formulations

This class of drugs was discovered as a result of the observation that sulfanilamide chemotherapeutic agents were capable of causing metabolic acidosis by inhibition of carbonic anhydrase (CA). Screening of sulfanilamides resulted in identification of compounds whose predominant mechanism of action was CA inhibition. These drugs have been used sparingly in veterinary medicine as diuretics and are more commonly used for ophthalmic purposes as topical formulations. The prototype drug in this class, acetazolamide (Diamox®, Dazamide®), is available in tablets (125 and 250 mg), extended-release capsules (500 mg), and injectable (500 mg per vial). Other CA inhibitors include preparations for oral use, dichlorphenamide (Daranide®) and methazolamide (Neptazane®), and a topical drug, dorzolamide (Trusopt®), for ophthalmic use.

Mechanisms and Sites of Action

Renal Mechanisms

Drugs in this class are active in the CA-rich segments of the nephron, in particular the proximal tubule. Noncompetitive, reversible inhibition of CA located in the luminal and basolateral membranes (type IV CA) as well as in the cytoplasm (type II CA) results in decreased formation of carbonic acid from CO_2 and H_2O (see Figure 24.5 and equation below):

$$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow H_2O + CO_2$$

Reduction in the amount of carbonic acid yields fewer H^+ within proximal tubular cells. Because H^+ is normally exchanged for Na^+ from the tubular lumen by the Na^+/H^+ antiporter (also referred to as a Na^+/H^+ exchanger or NHE), less Na^+ is reabsorbed and more is available to combine with urinary HCO_3^-. The NHE maintains a low proton concentration in the cell so that $H_2CO_3^-$ ionizes spontaneously to form H^+ and HCO_3^-. This, in turn, creates an electrochemical gradient for HCO_3^- across the basolateral membrane that drives movement of HCO_3^- into the interstitial space. Diuresis is established when water is excreted with sodium bicarbonate that accumulates due lack of CA activity. As sodium bicarbonate is trapped in the urine and eliminated, less HCO_3^- is returned to plasma, and a systemic acidosis eventually develops. As a result of the systemic acidosis, H^+ becomes available, Na^+ reabsorption is reestablished, and diuresis decreases. Continual use of CA inhibitors is therefore self-limiting in terms of diuretic action. Diuresis induced by CA inhibitors is mild due to incomplete inhibition of CA, redundancy of Na^+ transporting systems in the proximal tubule, and rescue of Na^+ by reabsorption later in the distal tubule. Because intracellular K^+ can, to some extent, substitute for H^+ in the Na^+ reabsorption step, CA inhibitors cause enhanced K^+ excretion. As more Na^+ is presented to the distal tubule, the potential for K^+ wasting increases. CA inhibitors also decrease secretion of titratable acids and ammonia in the collecting duct (Jackson, 1996). For this reason, and due to the increased excretion of sodium bicarbonate, urine pH increases despite the decreasing systemic pH associated with CA inhibitor induced acidosis. This class of drugs has little, if any, effect on excretion of Ca^{2+} and Mg^{2+} but does enhance phosphate elimination.

Extrarenal Actions

Other actions of CA inhibitors are related to the wide distribution of CA in body tissues including the eye, gastric mucosa, pancreas, central nervous system (CNS), and red blood cells. The most important therapeutic consequence is associated with CA inhibition in the eye. The ciliary processes of the eye mediate the formation of aqueous humor, which contains an abundance of osmotically active HCO_3^-. This process is CA dependent and, when inhibited, leads to a decreased rate of formation of aqueous humor and subsequent reduction in intraocular pressure. Although not therapeutically relevant in veterinary medicine, CA inhibition in the CNS has been associated with anticonvulsant actions attributed to this class of drugs.

Absorption and Elimination

Limited information is available regarding pharmacokinetics of CA inhibitors in animals. The pharmacokinetics of acetazolamide in dogs administered an extended release product produced an approximately 7-hour half-life with a T_{MAX} at 3 hours (Li et al., 2014). Acetazolamide gains access to the renal tubules via the organic acid secretion pathway. A dose of 22 mg/kg is reported to have an onset of action of 30 minutes, maximal effects in 2–4 hours, and a duration of action of 4–6 hours in small animals (Roberts, 1985). Oral absorption of drugs in this class is appears to be good in dogs. The oral bioavailability of acetazolamide is 25% in horses, with a terminal half-life of 7 hours (Alberts et al., 2000). Acetazolamide is eliminated primarily through the kidneys. The pharmacokinetics of methazolamide has not been reported in dogs, cats, or horses.

Toxicity, Adverse Effects, Contraindications, and Drug Interactions

Although CA inhibitors are sulfonamide derivatives, side effects commonly associated with sulfonamides are not reported or expected (Trepanier, 2004). CNS drowsiness and disorientation may occur as a result of inhibition of CA in the CNS. Because CA inhibitors decrease ammonia excretion, the severity of preexisting hepatic disease may be worsened and hepatic encephalopathy can be induced. Use is also contraindicated in patients with certain electrolyte disturbances (due to K^+ and Na^+ wasting) and those with metabolic or respiratory acidosis. Use in patients with severe pulmonary disease who cannot respond to drug-induced metabolic acidosis with respiratory compensation is also contraindicated. Because CA inhibitors alkalinize the urine, calcium phosphate calculi formation is enhanced, and excretion of weak organic bases is reduced. Rare blood dyscrasias associated with CA inhibitors have also been reported in the human, but not in the veterinary literature.

Therapeutic Uses

The primary indication for use of CA inhibitors is to inhibit production of aqueous humor and reduce intraocular pressure. Topical application of dorzolamide (q 8–12 h) produced similar reductions in intraocular pressure compared to oral methazolamide (5 mg/kg q 12 h) or the combination of topical and oral therapy (Gelatt and MacKay, 2001). The topical CA inhibitors therapy is preferred due to equivalent efficacy, lower potential for adverse effects, and lower cost than systemic administration. Acetazolamide (5–10 mg/kg PO q 8 h) and methazolamide (5 mg/kg PO q 12 h given two to three times daily) have been used previously in dogs for management of glaucoma. CA inhibitors have been administered to patients with hydrocephalus for short-term medical management, often in combination with furosemide, but long-term management with diuretics, in people, is often not successful (Thomas, 2010).

In human medicine, CA inhibitors have been used as an adjunctive therapy for epilepsy and in management of acute mountain (high-altitude) sickness. In both human and veterinary medicine, the use of CA inhibitors as diuretics has limited effectiveness due to the rapid development of tolerance. Theoretically, acetazolamide could be used to manage metabolic alkalosis, but this is not a typical clinical practice in veterinary medicine.

Osmotic Diuretics

Chemistry/Formulations

Osmotic diuretics contain simple solutes of low molecular weight that are typically freely filtered by the glomerulus, undergo limited tubular reabsorption, and are pharmacologically inert. They increase serum and tubular fluid osmolarity resulting in fluid shifts. The most common osmotic diuretic is mannitol, a six-carbon non-metabolized polyalcohol with a molecular weight of 182. Other agents include glycerin, isosorbide, urea, and hypertonic saline solutions. Because mannitol is the most commonly used osmotic diuretic in both human and veterinary medicine, subsequent discussion will focus primarily on this drug. Concentrated mannitol (15–25%) may crystallize at cooler temperatures, in which case the drug can often be resolubilized by warming the solution. Prior to administration, the solution should be cooled and any remaining crystals removed using an in-line intravenous (IV) filter. There are no veterinary-approved mannitol formulations available. For IV use in animals, the human formulations are used. Human products (Osmitrol®, Resectisol®) range from 5% (275 mOsm/l) to 25% (1375 mOsm/l) and are available for IV administration.

Mechanisms and Sites of Action

Hyperosmolar solutions exert part of their effects by establishing an osmotic gradient between plasma and extravascular fluid compartments resulting in fluid movement into the plasma. Acute effects of this gradient include decreases in hematocrit, blood viscosity, plasma sodium, plasma pH, and, to some degree, the volume of solid organs. Hence, parenchymal dehydration and acute hemodilution are theoretically related as long as the osmotically active particles are effectively separated by a relatively solute-impermeable barrier.

Renal Mechanisms

Initially, osmotic diuretics were thought to act primarily at the level of the proximal tubule by limiting the movement of water from the lumen into the interstitial space. Water retained in the tubular lumen diluted concentrations of sodium and other ions, reduced ion reabsorption, and promoted diuresis. It is now held that osmotic diuretics, in particular mannitol, have effects throughout the length of the tubule, with the most prominent action occurring in the loop of Henle. Sodium reabsorption is markedly reduced in the descending and thin limbs of the loop of Henle, as determined by studies in dogs and rats. Sodium load to the thick ascending limb of the loop of Henle and to the distal tubule is consequently increased, but the nephron fails to recapture the increased loads of salt and water. As demonstrated in the dog, sodium reabsorption is also thought to be directly inhibited in medullary collecting ducts (Better et al., 1997).

Other reported renal effects of mannitol include increases in cortical and medullary blood flow due to a decrease in renal vascular resistance, impairment of urinary concentration, reduction in medullary tonicity (also known as medullary washout), an increase in GFR during renal hypoperfusion (may vary according to species), and an increase in urinary excretion of other electrolytes (e.g., K^+, Ca^{2+}, Mg^{2+}, phosphate, bicarbonate) (Better et al., 1997). Mannitol may also prompt the release of atrial natriuretic factor and vasodilatory prostaglandins and inhibition of renin release.

Extrarenal Mechanisms

The actions of mannitol extend beyond the renal effects and include changes in blood rheology, direct transient effects on vascular tone, and increases in cardiac output. In addition to decreasing the hematocrit by hemodilution, mannitol decreases the volume, rigidity, and cohesiveness of red blood cell membranes. The combination of reduced viscosity and reduced mechanical resistance presumably leads to enhanced blood flow. Mannitol-induced increases in cardiac output are thought to be related to reduced peripheral resistance and reduced afterload, a transient increase in preload, and mild positive inotropy. Mannitol may also exert a cytoprotective effect by acting as an oxygen-free radical scavenger (Paczynski, 1997).

Absorption and Elimination

Mannitol is not metabolized and is handled as an inert substance by the body. Studies in dogs and humans indicate that mannitol distribution and elimination follow a two-compartment model (Cloyd et al., 1986; Rudehill et al., 1993). The distribution half-life of intravenously administered mannitol is measured in minutes. Elimination half-life is dose dependent and ranges from 0.5 to

1.5 hours for doses between 0.25 and 1.5 g/kg. Mannitol is eliminated rapidly by the kidneys unless renal function is impaired. As a result, penetration of mannitol into tissues is limited by rapidly falling plasma concentrations. Mannitol is administered intravenously in a slow bolus over 15–30 minutes. Glycerin and isosorbide are administered orally. Of the available osmotic diuretics, only glycerin is eliminated by biotransformation.

Adverse Effects and Drug Interactions

Acute Adverse Effects

Pulse pressure and mean arterial blood pressure usually increase transiently with mannitol administration. However, acute hypotensive, hyponatremic effects of mannitol administration have been reported, especially subsequent to rapid infusion in dehydrated individuals. The mechanism for this acute vasodilatory effect is not well understood, but the problem can largely be prevented by appropriate rates of administration (0.25–1.5 g/kg over 15–30 min). Acute hyponatremia may account for the nausea and vomiting that are sometimes observed with mannitol infusion. Rapid expansion of plasma volume related to attraction of fluid into the vascular compartment may precipitate CHF or pulmonary edema in certain patient populations. However, because the drug is cleared rapidly this problem is not common unless renal function is impaired or underlying cardiac disease is present.

Dehydration and Electrolyte Disturbances

Because the ratio of the volume of fluid eliminated in urine to the volume of mannitol administered is high, care should be taken to avoid hypertonic dehydration. Circulating plasma volume tends to be preserved as hypertonic dehydration develops, making it harder to clinically detect that a problem exists. The presence of dehydration and significant hypernatremia should be closely monitored using body weight, urine output, and other clinical parameters. In addition to hypertonic dehydration, loss of other electrolytes, including potassium, phosphate, and magnesium, can lead to clinically significant cardiac arrhythmias and neuromuscular complications.

Hyperosmolar State and Osmotic Compensation

The phenomenon of osmotic compensation occurs when cells respond to prolonged treatment with a hyperosmolar agent by increasing the presence of intracellular, idiogenic osmoles. Compensation is thought to occur rapidly when the osmolality of plasma is increased by 25 mOsm/kg or more above normal. Newly generated, osmotically active intracellular particles counteract the dehydrating effect of hyperosmolar plasma. Osmotic compensation can limit therapeutic effectiveness by decreasing the osmotic gradient from tissue to plasma. Increased intracellular osmolarity may also promote conditions, especially in the brain, where iatrogenic edema may occur. The risk of edema formation is increased if a hyperosmolar state is reversed rapidly, leaving the intracellular osmoles as the most osmotically active site. To prevent this complication, the duration of return of plasma to normal osmolality should be approximately equal to the duration of the hyperosmolar state.

Contraindications

The use of mannitol in patients with ongoing intracranial hemorrhage, anuric renal failure, severe dehydration, or pulmonary congestion or edema is contraindicated. Adequate fluid therapy should be administered to dehydrated animals prior to administration of mannitol. Mannitol should not be added to whole-blood products unless at least 20 mEq/l of sodium chloride is added to the solution; otherwise, pseudoagglutination may occur.

Therapeutic Uses

Mannitol is used in the prophylaxis and treatment of renal failure, for the reduction of intracranial and intraocular pressure, and with other diuretics to mobilize edema. For reasons already discussed, short-term use of mannitol is most effective to prevent adverse effects and decreased therapeutic efficacy.

Prophylaxis of Acute Renal Failure

Anuric patients should not be routinely treated with mannitol, although a small (0.25–0.5 g/kg), single test dose may be used to try and induce diuresis. Administration of mannitol to patients with renal dysfunction must be done cautiously to prevent problems associated with decreased elimination and prolonged hyperosmolarity. Acute renal failure (ARF) may be caused extrinsically (pre- and postrenal failure) or intrinsically, often associated with acute renal tubular necrosis (ATN). Mannitol has been found to be effective in limiting the decrease in GFR caused by ATN if administered before the ischemic insult or exposure to nephrotoxins. Protection of tubules from necrosis may be due to dilution of nephrotoxic substances, reduction of swelling of tubular elements, or removal of tubular casts that are obstructing urine flow. In human medicine, mannitol has been shown to be clearly beneficial in the preservation of kidneys for transplant and for decreasing the incidence of posttransplant ARF. Fewer data are available to support the general value of mannitol for treatment of ARF outside the area of transplantation (Better et al., 1997). In vascular and open-heart surgery, prophylactic mannitol maintains urine flow but not GFR. Some evidence suggests that mannitol administration in patients with established ATN may increase the conversion of oliguric to nonoliguric patients (Levinsky and Bernard, 1988).

Reduction of Intracranial Pressure

Osmotherapy has been used for decades to decrease intracranial pressure (ICP). Reduction in ICP is rapid and usually appears within minutes of completion of administration, with maximum effects within the hour. Several theories have been formulated to account for the effectiveness of mannitol in reducing ICP. The osmotic theory holds that brain shrinkage occurs as a result of osmotically driven movement of fluid from tissue and into the vascular compartment. Sensitive, high-resolution imaging methods seem to support the significance of osmotically induced changes in brain water content (Betz et al., 1989). The hemodynamic theory of ICP reduction states that cerebral blood volume is decreased as a result of decreased blood viscosity and increased cerebral perfusion pressure, both of which act to enhance oxygen delivery to the brain. Increased oxygen delivery to the brain is thought to trigger a compensatory reduction in vascular caliber and secondarily a reduction in cerebral blood volume. Detractors of this idea suggest that mannitol is just as likely to decrease blood viscosity as a result of hemoconcentration secondary to hypertonic dehydration. While attention to fluid replacement should prevent dehydration, it has recently been suggested that alternative agents, such as hypertonic saline, are safer and equally effective at reducing ICP (Raslan and Bhardwaj, 2007). Hypertonic saline has been shown to establish a strong transendothelial osmotic gradient but without the tendency to reduce intravascular volume (Prough and Zornow, 1998). Finally, the diuretic theory of ICP reduction suggests that mannitol-induced decreases in central venous pressure translate directly to decreases in ICP due to the valveless communication between the central venous system and the jugular drainage system. This effect may be more important in sustaining, rather than inducing, a decrease in ICP (Paczynski, 1997). Regardless of the theory, mannitol has been used for temporary reduction of ICP in patients with a variety of intracranial lesions as well as those with spinal cord trauma and edema. Evidence of ongoing intracranial hemorrhage is considered a contraindication for mannitol administration. Rebound increases in intracranial pressure have been observed with mannitol in 10–20% of human patients (Node and Nakazawa, 1990) and may be due in part to mannitol movement through the blood–brain barrier (Raslan and Bhardwaj, 2007).

Other Uses

Osmotic diuretics have also been used successfully to control intraocular pressure during acute glaucoma attacks and to reduce intraocular pressure before or after ophthalmic surgery. Decreases in intraocular pressure occur by loss of intraocular water to hyperosmolar plasma. As the vitreous shrinks, the lens moves posteriorly and the iridocorneal angle opens, improving drainage from the eye. The duration of action depends upon the degree to which the osmotic diuretic is excluded from ocular fluids. Mannitol is reported to increase retinal oxygen tension and is used at a dose of 1–2 g/kg at a rate of 1 ml/kg/min to reduce intraocular pressure in dogs. Although still useful for decreasing intraocular pressure due to glaucoma, other therapies including topical carbonic anhydrase inhibitors (i.e., dorzolamide), β_1 antagonists (i.e., timolol), and prostaglandin analogues (i.e., latanoprost) are the preferred therapies.

Inhibitors of $Na^+/K^+/2Cl^-$ Symport (Loop or High-Ceiling Diuretics)

Drugs belonging to this class are among the most effective and the most commonly prescribed diuretics, and all share a common mechanism of action. By blocking a key sodium transport mechanism in the thick ascending limb of the loop of Henle (hence the name loop diuretic), these drugs inhibit reabsorption of up to 25% of the filtered sodium load. Nephron segments distal to the thick ascending limb (TAL) are incapable of reabsorbing the additional solute, leading to a marked (or high-ceiling) natriuresis and diuresis. Because furosemide (Salix®, Disal®) is by far the most commonly used diuretic in veterinary medicine, the remaining discussion will focus primarily on this drug (also referred to as frusemide). Other drugs in this class include ethacrynic acid (Edecrin®), bumetanide (Bumex®), and the most recent addition approved in the United States, torsemide (Demadex®).

Chemistry/Formulations

Except for ethacrynic acid, the structurally diverse drugs in this class are sulfonamide derivatives; however, they are not subject to the same adverse effects as sulfonamide antimicrobials. Furosemide is light sensitive and stable under alkaline conditions; the veterinary injectable preparations (Salix or generic; 50 mg/ml) have a light yellow color, whereas the human injectable (Lasix®; 10 mg/ml) should not be used if it appears yellow. A wide range of veterinary preparations of furosemide are available, including oral tablets approved for use in dogs and cats (12.5 mg, 50 mg), oral solution approved for use in dogs (10 mg/ml), large-animal boluses approved for use in cattle (2 g/bolus), and injectable (5%) approved for use in dogs, cats, horses not intended for food, and cattle.

Milk and slaughter withdrawal time for both oral and injectable formulations for cattle is 48 hours. Bumetanide (Bumex® is supplied in 0.5, 1, and 2 mg tablets and as a 0.25 mg/ml parenteral injection. Bumetanide is approximately 40 to 50 times more potent than furosemide in the dog. Torsemide (also torasemide) (Demadex) is supplied as 5, 10, 20, and 100 mg tablets and in 2 or 5 ml ampules containing 10 mg/ml for intravenous administration. Torsemide dosage in the dog has been estimated at approximately one-tenth the human dose but the drug has not been well studied in dogs and cats (Kittleson and Kienle, 2007).

Mechanisms and Sites of Action

Renal Effects

Drugs in this class block the Na^+-K^+-$2Cl^-$ symporter in the TAL by binding to the Cl^- binding site of the transporter protein (Figure 24.6). All of these drugs must be actively secreted into the tubular lumen by an organic acid pathway in order to reach and inhibit the luminal symporter. A high degree of protein binding (>95%) limits glomerular filtration of furosemide and other loop diuretics, making tubular secretion essential.

The mechanism involved in Na^+ reabsorption in the TAL depends upon Na^+/K^+ ATPase activity in the basal membrane of tubular cells, creating an electrochemical gradient. The low intracellular sodium concentration induced by the Na^+/K^+ATPase drives the absorption of sodium, along with potassium and chloride, from the tubular fluid by $Na^+/K^+/2Cl^-$ symporter located in the luminal membrane. Recycling of the potassium back into the tubular fluid is accomplished by the potassium channels on the luminal membrane. Chloride channels on the basolateral side efflux chloride from the intracellular compartment. Basolateral Cl^- conductance and luminal K^+ conductance determine membrane voltage. The polarity of K^+ and Cl^- conductances results in a lumen-positive transepithelial voltage. This voltage drives cations (Ca^{2+} and Mg^{2+}) between tubular cells via the paracellular shunt pathway. When loop diuretics block the $Na^+/K^+/2Cl^-$ symporter, Cl^- concentrations in the cell fall, the cell becomes hyperpolarized, transepithelial voltage is disrupted, and paracellular cation reabsorption is blocked (Bleich and Gregor, 1997). Because renin-producing cells in the area of the macula densa generate part of their membrane voltage via Cl^- channels, the new Cl^- equilibrium also depolarizes these cells and enhances the secretion of renin.

Loop diuretics interfere with establishment of a hypertonic medullary interstitium (due to impaired sodium reabsorption) and disrupt the countercurrent mechanism. Hence, these drugs block the kidney's ability to concentrate and dilute urine appropriately. Inhibitors of the $Na^+/K^+/2Cl^-$ symporter also inhibit Ca^{2+} and Mg^{2+} reabsorption in the TAL by disruption of the transepithelial potential difference. Some loop diuretics, notably furosemide, also have weak carbonic anhydrase-inhibiting activity that leads to enhanced urinary excretion of HCO_3^- and phosphate. All $Na^+/K^+/2Cl^-$ symporter inhibitors increase the urinary excretion of K^+ and H^+ by presenting a greater load of Na^+ to the distal tubule. Sodium is reabsorbed while K^+ and H^+ are excreted.

Nonsteroidal antiinflammatory drugs (NSAIDs) inhibit the diuretic, natriuretic, and chloruretic responses to furosemide. Furosemide enhances production of prostaglandin E2 (PGE_2), which in turn inhibits chloride and sodium reabsorption in the TAL. In the presence of NSAIDs, PGE_2 production is blocked, and furosemide-induced diuresis is decreased (Kirchner, 1987). In the absence of volume depletion, loop diuretics generally increase and redistribute total renal blood flow. The mechanism for this effect is thought to be related to prostaglandins, and the effect is diminished or blocked in the presence of NSAIDs (Data et al., 1978). Furosemide-induced hemodynamic changes correlate with an increase in urinary excretion of PGE_2.

Extrarenal Effects

Furosemide causes extrarenal hemodynamic effects that include increased venous compliance and decreased right atrial pressure, decreased pulmonary artery pressure, decreased pulmonary artery wedge pressure, and decreased pulmonary blood volume (Hinchcliff and Muir, 1991). Prostaglandins are thought to account for the acute increase in systemic venous capacitance and subsequent decrease in left ventricular filling pressure. All of these effects are dependent upon the presence of a functional kidney and the uninhibited production of prostaglandins. In the isolated rabbit heart, furosemide has also been reported to exert a mild negative inotropic effect that is prostaglandin dependent (Feldman et al., 1987). Similarly, prostaglandins are important for furosemide's effects in horses since phenylbutazone decreased its diuretic and vascular responses (Hinchcliff et al., 1995).

Inhaled furosemide in humans has been shown to protect against the early response to inhaled allergens and to prevent exercise-induced bronchoconstriction (Bianco et al., 1988, 1989). Furosemide may prevent bronchoconstriction in part by inhibiting release of inflammatory mediators from lung cells (Anderson et al., 1991). Pulmonary gas exchange is reportedly improved by furosemide in experimental pulmonary edema. Furosemide also reduces the rate of pulmonary transvascular fluid filtration through a reduction in pulmonary vein pressure (Demling and Will, 1978).

Absorption and Elimination

Furosemide is approximately 77% bioavailable in dogs and has an elimination half-life of about 1 hour following an IV dose of 5 mg/kg (Hirai et al., 1992). The absorption of orally administered furosemide takes place mainly in the upper parts of the canine gastrointestinal tract, decreasing rapidly across the jejunum. Peak diuretic effects of an IV dosage of furosemide in the dog are reported at approximately 30 minutes and following oral dosing at about 1–2 hours. The rate of urinary furosemide excretion, more so than the concentration of plasma furosemide, has been found to closely correlate with diuretic response in dogs. As in humans, the relation between the natriuretic response and the concentration of diuretic in the urine (at the site of action) is represented by a typical sigmoidal concentration–response curve. The shape of the curve suggests that a threshold quantity of drug must be achieved at the site of action in order to elicit a response and that a maximal dose can be identified that yields a maximal response. Beyond that maximal dose, the curve plateaus, and limited additional benefits are derived from dose increases (Brater, 1998; Hirai et al., 1992). Because intermittent doses produce a brief effect in dogs and higher doses do not produce a greater effect, the use of a constant-rate infusion (CRI) has been explored. In dogs, a loading dose of 0.66 mg/kg is followed by a CRI of 0.66 mg/kg/h × 8 hours. This results in greater natriuresis, calciuresis, and diuresis than with intermittent treatment (Adin et al., 2003).

Elimination half-life of IV furosemide in the horse is similar to that in the dog and, in the absence of renal impairment, is slightly less than 1 hour. Furosemide is typically administered IV or IM three to four times daily to horses. A study comparing oral and IV administration of the drug determined that systemic availability of furosemide given orally is poor, erratic, and variable among horses. Median systemic bioavailability was low and, at a dose of 1 mg/kg PO, diuresis was not induced (Johansson et al., 2004). In a study comparing CRI to intermittent administration of furosemide in the horse, CRI of the drug produced more uniform urine flow, decreased fluctuations in plasma volume, and suppressed renal concentrating ability throughout the study. Although potassium, calcium, and chloride excretion were higher with CRI than intermittent administration, CRI was preferred if profound diuresis was required in the horse (Johansson et al., 2003).

In humans and other animals, including the dog and horse, approximately 50–60% of a furosemide dose is excreted unchanged in the urine, and the remaining drug is conjugated to glucuronic acid in either the kidney, the liver, or other extrahepatic site (Brater, 1998; Dyke et al., 1998).

Plasma half-life in patients with renal insufficiency is prolonged, and dosage adjustments should be made. Binding of furosemide to excessive amounts of albumin (>4 g/l) in the urine decreases the amount of unbound, active drug and diminishes the diuretic response. In human patients with nephrotic syndrome, doses of two to three times normal are recommended to provide sufficient amounts of active drug to block the $Na^+/K^+/2Cl^-$ symporter.

In humans, bumetanide and torsemide are metabolized in large part by the liver, and so dosage generally does not need to be adjusted for renal disease. Bumetanide's potency in the dog is, in part, explained by limited biotransformation. In addition, renal uptake of bumetanide is greater than furosemide and the drug has a more marked effect on sodium transport in the ascending limb of the Loop of Henle. Approximately 67% of bumetanide in dogs is eliminated unchanged in the urine and feces (Schwartz, 1981). Oral bioavailability of these drugs is much more predictable than that of furosemide and in humans ranges from 80 to 100% (Brater, 1998).

Toxicity, Adverse Effects, Contraindications, and Drug Interactions

Most adverse effects of furosemide administration are related to abnormalities of fluid and electrolyte balance. Extracellular volume depletion and hyponatremia may lead to reduced blood pressure and diminished organ perfusion. Most at risk for adverse effects related to volume depletion are patients with renal disease (may decrease GFR, increase prerenal azotemia, and, possibly, cause tubular necrosis), cardiac disease (stroke volume and cardiac output may decrease), and hepatic disease (precipitation of hepatic encephalopathy). As noted previously, $Na^+/K^+/2Cl^-$ symporter inhibitors deliver an increased load of Na^+ to the distal tubules, resulting in a renin–angiotensin–aldosterone-driven increase in excretion of K^+ and H^+ in exchange for Na^+. Hypochloremic alkalosis and hypokalemia may result. Risk factors for cardiac dysrhythmias related to diuretic-induced hypokalemia include inadequate dietary intake of K^+, concurrent administration of cardiac glycosides, and additional electrolyte imbalances. A common cause of anorexia in CHF patients is digitalis toxicity, and risk of arrhythmias is increased in these patients if hypokalemia is present. Deficiencies in Mg^{2+} and Ca^{2+} may also be caused by diuretic-enhanced excretion of these substances. Serum electrolyte levels should be monitored in patients receiving ongoing diuretic therapy, especially if risk factors exist related to appetite, diuretic dosage, or severity of disease.

Ototoxicity, which is usually transient, has been described primarily with ethacrynic acid and less often with all other loop diuretics. In veterinary medicine, ototoxicity may be of greatest concern in treatment of cats with high-dose IV regimens. Other adverse effects reported with use of loop diuretics include gastrointestinal disturbances, bone marrow depression, and hyperglycemia. Hyperglycemia may be related to impairment of proinsulin-to-insulin conversion associated with diuretic-induced decreases in K^+ levels. Patients hypersensitive to sulfonamides may also be hypersensitive to furosemide since this drug contains a sulfonamide moiety. Diuretic-induced depletion of water-soluble vitamins may occur, and supplementation of B-complex vitamins has been recommended for animals receiving continuous diuretic therapy (Keene and Rush, 1995).

Loop diuretics are contraindicated in animals with severe fluid and electrolyte disturbances or anuria that does not respond to test doses of diuretics.

Drug interactions may occur when furosemide is administered with theophylline/aminophylline (enhanced effects due to theophylline-induced diuresis), aminoglycosides or cisplatin (enhanced ototoxicity and, if volume depleted, nephrotoxicity), digitalis glycosides (diuretic-induced hypokalemia may increase risk of arrhythmias), aspirin or other anticoagulants (anticoagulant activity increased), neuromuscular blockers (alteration in extent of muscle relaxation), corticosteroids (enhanced potassium wasting), insulin (alteration of insulin requirements associated with hyperglycemic effects), lithium and propranolol (increased plasma levels), probenecid (competition for secretion of diuretic into tubular lumen leading to decreased diuretic effect), NSAIDs (as previously described, decreased diuretic effects), and thiazides (synergistic diuretic activity).

Therapeutic Uses

Furosemide is used in small animals for treatment of edema of cardiac, hepatic, or renal origin. In general, the dose of drug in dogs (1–3 mg/kg every 8–24 hours PO for chronic use; 2–5 mg/kg every 4–6 hours IV, IM, SC) is higher than that used in cats (1–2 mg/kg every 12 hours up to 4 mg/kg every 8–12 hours IV, IM, SC, PO) (Ware, 1998). Furosemide is also used to establish diuresis in renal failure and to promote excretion of other substances, including elevated electrolytes such as Ca^{2+} and K^+. In large animals, furosemide has been used to treat edema in cattle and edema and exercise-induced pulmonary hemorrhage (EIPH) in horses. A general dose of 0.5–1 mg/kg twice daily or as needed to control edema has been recommended in large animals (Reef and McGuirk, 1996). Benefits of furosemide use for treatment of EIPH remain controversial (see Section Exercise-Induced Pulmonary Hemorrhage). Specific state guidelines should be consulted for details of furosemide use (dose, frequency, allowable levels) in racing animals.

Diuretic effects of orally administered torsemide (0.3 mg/kg) and furosemide (3 mg/kg) were compared in dogs and cats (Uechi et al., 2003). Both furosemide and torsemide increased urine volume but the effects of furosemide peaked at 2–3 hours and dissipated by 6 hours while the effects of torsemide peaked at 2–4 hours but persisted for 12 hours in normal dogs, dogs with mitral regurgitation, and cats with experimentally induced left ventricular concentric hypertrophy. It was noted that torsemide decreased urine potassium excretion in study dogs with mitral regurgitation. In a previous study, the ratio of sodium to potassium excretion was found to be 20:1 for torsemide and 10:1 for furosemide (Ghys et al., 1985).

Renal Insufficiency

Decreased GFR and decreased delivery of drug to the tubular site of action and site of elimination in renal insufficiency results in decreased efficacy and increased half-life of furosemide. A sufficiently high dose of drug must be administered to attain an effective amount of drug at the site of action. For the dog, furosemide doses starting at 2 mg/kg IV and increasing in 2 mg/kg increments every hour for 3 hours may be used to try and induce diuresis in severe renal insufficiency. Once a maximal dosage is reached (approximately 6–8 mg/kg), exceeding this amount is not advantageous based on the sigmoidal shape of the fractional sodium excretion curve. In all cases, fluid deficits should be addressed prior to furosemide therapy.

Cardiogenic or Pulmonary Edema

Furosemide has been widely used to reduce extracellular volume and minimize venous and pulmonary congestion in chronic and acute CHF. In humans, beneficial effects in congestive heart failure are noted prior to induction of diuresis suggesting vascular effects are important components of furosemide benefits in patients with pulmonary edema. Human patients with CHF do not require large dosages since furosemide is adequately delivered to the tubular fluid. However, because renal responsiveness to loop diuretics appears to be decreased in these patients, increased frequency of administration has been recommended (Brater, 1998). Diuretics have traditionally been considered front-line therapy for the treatment of chronic CHF in small animals, and furosemide is reported to be the most frequently used drug for this purpose (Goodwin and Hamlin, 1993; Watson and Church, 1995). Despite the popularity of furosemide, human studies have revealed that CHF patients controlled on loop diuretics alone deteriorate more quickly than those treated concurrently with either angiotensin-converting enzyme (ACE) inhibitors or digoxin. Use of furosemide alone is thought to enhance early activation of the renin–angiotensin–aldosterone system, with detrimental effects on long-term prognosis (Swedberg et al., 1990). Current recommendations include furosemide for treatment of more advanced stages of heart failure in patients already receiving ACE inhibitors, digoxin, or both. Furosemide remains a drug of choice for treatment of acute cardiogenic pulmonary edema. Within the context of severity and chronicity of disease, the lowest effective dosage and frequency of furosemide administration should be determined by observation of clinical signs and consideration of owner observations.

Exercise-Induced Pulmonary Hemorrhage

Exercise-induced pulmonary hemorrhage**,** or bleeding from the lungs as a consequence of intense exercise, occurs in horses engaged in a variety of athletic activities. The problem has been best studied in racing horses, particularly Thoroughbreds. A consensus statement on EIPH has been released by the American College of Veterinary Internal Medicine (Hinchcliff et al.,2015).

In most studies, furosemide has been found to reduce right atrial, pulmonary arterial, and pulmonary wedge pressures in exercising horses. Some studies suggest that changes in pulmonary pressures caused by furosemide are due to reduction in plasma and blood volume and not to direct effects of the drug on the pulmonary vasculature. Furosemide produces a rapid reduction in blood and plasma volume, which has been shown in the horse to be essential for subsequent reduction in pulmonary pressures (Hinchcliff et al., 1996). Furthermore, administration of polyionic fluids in an amount equal to the volume lost in urine restores furosemide-induced decreases in right atrial pressure and blood volume in the horse (Rivas and Hinchcliff, 1997). Furosemide administration 4 hours prior to exercise has been found to significantly decrease pulmonary capillary hypertension, which is thought to correlate with decreased risk of EIPH. Administration of the drug at shorter intervals prior to exercise did not result in more effective attenuation of exercise-induced pulmonary capillary hypertension (Magid et al., 2000). Combination of furosemide with either clenbuterol (Manohar et al., 2000) or pentoxifylline (Manohar, 2001) have not been found to enhance the pulmonary hemodynamic efficacy of furosemide in EIPH.

Data from additional studies leave open the question of direct effects of furosemide on pulmonary pressures and mechanics in EIPH. In one study horses treated with NSAIDs (phenylbutazone and flunixin) prior to administration of furosemide followed by exercise did not show reductions in pulmonary and right atrial pressures (Olsen et al., 1992). In a subsequent study these effects could not be reproduced (Manohar, 1994). Differences in the studies may be related to drug dosages, time of administration, amount of diuresis, and degree of cyclooxygenase inhibition. While it is accepted that NSAIDs decrease the diuretic and vascular responses to furosemide, it is as yet unresolved whether these drugs mitigate furosemide-induced reductions in pulmonary and right atrial pressures. It is also not clear whether the magnitude of reduction of pulmonary capillary transmural pressure with furosemide is sufficient to prevent capillary rupture in exercising horses (Soma and Uboh, 1998). This is consistent with clinical observations that furosemide reduces, but does not completely eliminate, pulmonary hemorrhage in exercising horses.

Administration of furosemide to racing animals is thought to enhance their performance, although this conclusion remains somewhat controversial. Use of furosemide in racing Thoroughbreds, Quarter Horses, and Standardbreds is estimated at 74.3, 19, and 22.5%, respectively (Hinchcliff, 1999). A cross-sectional study concluded that Thoroughbreds receiving furosemide raced faster, earned more money, and were more likely to win or finish in the top three positions than unmedicated horses (Gross et al., 1999). Early studies showed increases in racing times when EIPH was diagnosed and a subsequent improvement of racing times upon administration of furosemide (Soma et al., 1985). Treadmill studies have not consistently shown furosemide-induced changes in maximal O_2 consumption, time to fatigue, or the speed at which fatigue occurred in exercising horses (Hinchcliff et al., 1993). However, in these and later studies, the loss of weight associated with furosemide administration did reduce carbon dioxide production, the respiratory exchange ratio, and plasma lactate. Furosemide-induced gains in performance were reversed by addition of a weight equal to the weight of the volume lost. These results suggest that performance benefits associated with furosemide administration to EIPH horses may be unrelated to reduction in hemorrhage and more related to changes in body weight (Soma and Uboh, 1998). Based on human studies, this interpretation should not be extended to circumstances in which the race distance is long and exertion prolonged. In these cases, the detrimental effects of dehydration would rapidly offset the advantage of running under reduced weight.

A final issue related to use of furosemide in racing animals involves the regulation of administration of furosemide and other drugs to equine athletes. The control of drugs in racing animal is discussed in more detail in Chapter 57. Doses of 250–500 mg furosemide per horse (0.5–1.0 mg/kg) administered IV no later than 4 hours prior to post time are permitted for medication of horses with EIPH in most jurisdictions in the United States. Specific regulations at a given track should be consulted. The regulation of furosemide administration according to track rules has been approached in a variety of ways. Some jurisdictions use a combination of urine specific gravity of 1.015 or 1.010 and a plasma concentration of greater than 60 or 100 ng/ml as an indication of a violation of the rules. The combination of these two parameters, low specific gravity and high plasma concentration, will suggest that an irregularity related to dose, time, or route of furosemide administration occurred (Soma and Uboh, 1998). By considering both urine specific gravity and plasma furosemide concentration, the probability of misclassifying horses as being in violation of regulatory concentrations is reduced (Chu et al., 2001).

Widespread use of furosemide in racing animals also presents problems related to screening of urine for presence of regulated substances. The urinary concentration of coadministered drugs may be diluted as a function of furosemide-enhanced diuresis. Urinary excretion rates of some drugs, especially those that are water-soluble acids, may be altered as a result of furosemide competition for the organic anion tubular secretion pathway. Furosemide has been shown to decrease the urinary concentration of phenylbutazone through both of these mechanisms. In comparison, the excretion rate of other agents, notably fentanyl, procaine, and methylphenidate, is increased by furosemide. Faster clearance of these substances may make it more difficult to detect illegal use prior to a race (Hinchcliff and Muir, 1991).

Other Uses

Furosemide has been shown to decrease pulmonary resistance and increase dynamic compliance in ponies with chronic obstructive pulmonary disease. In this case, the rapidity of the response and the finding that the response could be blocked by NSAIDs suggested a cyclooxygenase-mediated event rather than an effect dependent upon loss of body fluid (Broadstone et al., 1991). Immediate changes in pulmonary pressures in other species (e.g., dogs with pulmonary edema) are thought to be related to direct effects of furosemide on the pulmonary vasculature. Similar to use in small animals, furosemide is indicated for treatment of CHF and associated pulmonary edema by decreasing cardiac preload and plasma volume. Furosemide is also recommended to increase urine flow in acute renal failure in horses.

Inhibitors of Na^+/Cl^- Symport (Thiazide and Thiazide-Like Diuretics)

Chemistry/Formulations

Thiazide diuretics are benzothiadiazines or analogs and are derivatives of CA-inhibiting sulfonamides. Compared to carbonic anhydrase inhibitors which promote the elimination of sodium bicarbonate, thiazides promote renal excretion of sodium chloride producing a true saluretic effect. Two of the first thiazides synthesized, and the two drugs most commonly used in veterinary medicine, are chlorothiazide (Diuril®, human-approved 250 and 500 mg tablets, 50 mg/ml suspension, and 500 mg/vial injectable available) and hydrochlorothiazide (Hydrozide®, veterinary-approved 25 mg/ml injectable; HydroDiuril®, human-approved 25, 50, and 100 mg tablets and 10 mg/ml oral suspension). Both drugs are derivatives of benzothiadiazine and are water soluble. Hydrozide® is the only veterinary-approved product for use in cattle and has a 72-hour milk withholding time for lactating dairy cattle; no meat withholding time has been reported. Newer generation, more lipid-soluble benzothiadiazine derivatives include cyclothiazide and methychlothiazide.

Nonbenzothiadiazine derivatives have thiazide-like effects, and these drugs also promote excretion of sodium with chloride. Quinazolinone derivatives are in this class and include metolazone and chlorthalidone. These drugs are not commonly used in veterinary medicine but are examples of thiazide-like diuretics.

Mechanisms and Sites of Action

The primary site of action of thiazides is the distal convoluted tubule, with some secondary activity, possibly CA-related, in the proximal tubule. In the distal tubule, Na/Cl reabsorption is mediated by an electroneutral cotransport (symport) system (Figure 24.9). The driving force for Cl^- entry is the transmembrane Na^+ gradient established by the activity of basolateral Na^+/K^+-ATPase. The apical (luminal) Na/Cl cotransporter is reversibly inhibited by thiazides. Basolateral movement of Cl^- out of the cell is mediated by a Cl^- channel and K^+ by a K^+ channel. The lumen-negative transepithelial potential generated by the polarity of K^+ and Cl^- exit may drive anion reabsorption via a paracellular shunt pathway. Ca^+ reabsorption is enhanced by thiazides, by increasing distal tubule Na^+/Ca^{2+} exchange (antiporter) on the basolateral membrane due to low intracellular sodium. Because 90% of filtered Na^+ is reabsorbed prior to the distal tubule, the peak diuresis caused by thiazides is moderate compared to loop diuretics. Like loop diuretics, thiazides enhance excretion of K^+ by increasing the delivery of Na^+ to the distal tubule.

Absorption and Elimination

Thiazide and thiazide-like diuretics are absorbed slowly and incompletely from the gastrointestinal tract. Most drugs in this class are highly protein bound and undergo renal excretion (chlorothiazide and hydrochlorothiazide) or by a combination of renal and biliary routes (thiazide-like drugs). Hydrochlorothiazide is less protein bound (40%) than others in the class and partitions and accumulates in red blood cells (Velazquez et al., 1995). All drugs in this class gain access to the lumen of the renal tubule via an organic acid secretory pathway. Hence effectiveness of these drugs is decreased if renal blood flow diminishes.

Toxicity, Adverse Effects, Contraindications, and Drug Interactions

Similar to loop diuretics, most problems associated with administration of thiazides are related to fluid and electrolyte disturbances. Potassium wasting, especially with concurrent use of digitalis, increases the risk of cardiac arrhythmias. Low K^+ may secondarily affect conversion of proinsulin to insulin, leading to hyperglycemia. Enhanced calcium reabsorption can lead to hypercalcemia, and mild magnesuria may cause magnesium deficiency. Depletion of extracellular volume, hyponatremia, hypochloremia, and hypochloremic metabolic alkalosis may occur as adverse effects with prolonged or aggressive thiazide use. Because thiazides block solute reabsorption at nephron sites involved in dilution of urine, these agents increase the risk of hyponatremia under conditions of increased consumption of hypotonic fluids. CNS and gastrointestinal effects may occur but are not common.

Although thiazides contain sulfur, they do not induce reactions similar to sulfa antimicrobials (Trepanier, 2004). Patients with severe renal disease, hypovolemia, or electrolyte disturbances are poor candidates for thiazide therapy. Impaired hepatic function that may be worsened by volume contraction (leading to hepatic encephalopathy) is a contraindication for thiazide use. Diabetic patients are at risk for thiazide-induced derangements of glucose and insulin.

Drug interactions may include decreased effects of anticoagulants and insulin and increased effects of some anesthetics, diazoxide, digitalis glycosides, lithium, loop diuretics, and vitamin D. Combination therapy using low-dose thiazides with antihypertensives (e.g., ACE inhibitors) is currently considered to be an effective alternative strategy for management of human hypertension (Neutel et al., 1996). At low doses, adverse effects of thiazides are decreased, making their use in combination regimens particularly appealing.

Thiazides are reported to prolong the half-life of quinidine. In the face of thiazide-induced hypokalemia, an elevated plasma quinidine level increases the risk of polymorphic ventricular tachycardia (torsades de pointes), a condition that can deteriorate into ventricular fibrillation (Jackson, 1996). NSAIDs may reduce the effectiveness of thiazides and loop diuretics (Brater, 1998).

Therapeutic Uses

Thiazides may be used to treat edema of cardiac, hepatic, or renal origin. Typical oral dosages in the dog and cat are 20–40 mg/kg every 12 hours (chlorothiazide) and 2–4 mg/kg every 12 hours (hydrochlorothiazide). Effects of both chlorothiazide and hydrochlorothiazide peak at 4 hours and last up to 12 hours, with hydrochlorothiazide typically having a longer duration (12 hours) than chlorothiazide (6–12 hours). Cattle may be treated for udder edema with hydrochlorothiazide (125–250 mg IV or IM once or twice daily). Oral chlorothiazide (not a veterinary-approved product) at a dose of 4–8 mg/kg once or twice daily has been substituted for injectable hydrochlorothiazide following the first or second day of parenteral treatment.

Thiazides have previously been used in veterinary medicine in management of the early stages of CHF. As mentioned previously, early use of loop and thiazide diuretics in CHF activates aldosterone-mediated mechanisms that eventually lead to cardiac deterioration. For this and other reasons, the use of thiazides in treatment of CHF is not common in veterinary medicine. In general, furosemide is more commonly used in veterinary medicine to treat edema, whether it be cardiac, hepatic, or renal in origin. In human medicine, thiazides are commonly used in the management of hypertension. Because thiazides increase reabsorption of calcium, they may also be beneficial in treatment of calcium nephrolithiasis in humans and animals.

Thiazides are used effectively to reduce the volume of urine in patients with nephrogenic diabetes insipidus. Diuretic-induced volume contraction leads to increased proximal tubule reabsorption and a decrease in urine volume of 30–50%. Although dosages are individualized in these patients, starting ranges of 10–20 mg/kg twice daily (chlorothiazide) or 2.75–5.5 mg/kg (hydrochlorothiazide) twice daily have been suggested (Nichols and Thompson, 1995).

Inhibitors of Renal Epithelial Sodium Channels (K^+-Sparing Diuretics)

Chemistry/Formulations

The two relevant drugs in this class, triamterene (Dyrenium®) and amiloride (Midamor®), both belong to the class of cyclic amidine diuretics. Triamterene is a pteridine ring with amino groups at the 2, 4, and 7 positions. It was originally synthesized as a folic acid antagonist. Amiloride consists of a substituted pyrazine ring with a carbonylguanidinium side chain. A number of analogs of this basic structure have been synthesized and have been useful tools in elucidating mechanisms of sodium transport. Both triamterene and amiloride are organic bases and are secreted into the proximal tubule by an organic base transport system. Although neither of these drugs is used with frequency in veterinary medicine, triamterene is the more commonly used and hence will be the focus of these discussions. No parenteral forms of the drug are available; oral preparations are available in 50 and 100 mg capsules.

Mechanisms and Sites of Action

Triamterene and amiloride cause a mild increase in excretion of NaCl and a retention of K^+. Both drugs slightly augment diuresis and are used in combination with loop diuretics or thiazides to decrease K^+ excretion (hence the term K^+-sparing). Both drugs act at the late distal tubule (or connecting tubule) and collecting duct to block the electrogenic transport of Na^+. As with other diuretics, the basolateral Na^+/K^+-ATPase creates an electrochemical gradient that drives events at the luminal surface of the tubular cell. In this case, the principal cells of the connecting tubule contain a Na^+ channel in their luminal membrane that provides a pathway for entry of Na^+ and sets up a lumen-negative transepithelial potential. The transepithelial voltage is the key force involved in driving K^+ out of the principal cell and into the tubular lumen. Blockade of Na^+ channels by triamterene or amiloride hyperpolarizes the luminal membrane, reduces the lumen–negative potential difference, and decreases the excretion of K^+, H^+, Ca^{2+}, and Mg^{2+}. It has been speculated that effects of both of these drugs may also be mediated by inhibition of a Na^+/H^+ antiport located in the late distal tubule and collecting duct. Additional, direct effects on Mg^{2+} excretion may also occur.

Triamterene has been shown to exert cardiac effects that are not secondary to alterations in renal function. Early studies documented a prolongation of the cardiac action potential duration and functional refractory period and an increase in myocardial contractile force. Triamterene has also been shown to decrease digitalis-induced K^+ loss from the heart and increase the dose of digitalis necessary to induce toxic effects in dogs (Palmer and Kleyman, 1995; Netzer et al., 1995). Neither triamterene nor amiloride has been shown to affect renal hemodynamics or glomerular filtration rates, and neither acts as an aldosterone antagonist.

Absorption and Elimination

Both amiloride and triamterene are administered orally; triamterene is up to 70% bioavailable, but neither drug has been extensively evaluated in most veterinary species. Amiloride is renally excreted. The pharmacokinetics of triamterene are complex. The parent drug is converted in the liver to an active metabolite, 4-hydroxytriamterene sulfate, which is actively secreted into the renal tubules. Hence renal or hepatic disease could impair elimination of triamterene. The peak onset of action of triamterene is 6–8 hours, with effects persisting up to 12–16 hours.

Toxicity, Adverse Effects, Contraindications, and Drug Interactions

The most important potential adverse effect of these drugs is hyperkalemia. The presence of diseases or circumstances that may increase the risk of hyperkalemia (e.g., renal failure, coadministration of other drugs with K^+-sparing properties, including ACE inhibitors and K^+ supplements and NSAIDs) should be noted and these patients treated with other diuretic combinations. Triamterene may decrease GFR and, in combination with NSAIDs, has been shown to increase the likelihood of hyperkalemia and renal dysfunction. Triamterene-induced renal casts may be responsible for increased risk of interstitial nephritis and renal stones. Both triamterene and amiloride may induce hypersensitivity reactions that include rash and photosensitivity in humans. CNS, gastrointestinal, and hematological side effects have also been reported. As with most other diuretics, use in patients with severe hepatic disease or renal disease is contraindicated. In human patients with hepatic disease, the mild folic acid antagonism inherent in triamterene may increase the risk of megaloblastosis.

Therapeutic Use

Because these drugs have relatively weak diuretic properties, they are clinically important primarily because of their K^+-sparing properties in combination with loop and thiazide diuretics. Both have been used in this capacity for treatment of edema associated with CHF, liver cirrhosis, nephrotic syndrome, steroid-induced edema, and idiopathic edema. Triamterene is administered at a dose of 2–4 mg/kg/day orally to dogs with food to avoid gastrointestinal side effects.

Antagonists of Mineralocorticoid Receptors (Aldosterone Antagonists and K^+-Sparing Diuretics)

Chemistry/Formulations

Spironolactone is a 17-spirolactone and along with eplerenone are the only aldosterone antagonists approved in the United States. Canrenone, an active metabolite of spironolactone, and potassium canrenoate are closely related structurally and are available in other countries. All of these drugs share a four-ring, steroid structure similar to the mineralocorticoid aldosterone. Spironolactone is available as a human-approved oral preparation (Aldactone®) in 25, 50, and 100 mg tablets and eplerenone is available in 25 and 50 mg tablets.

Mechanisms and Sites of Action

Aldosterone is a steroid hormone that binds to mineralocorticoid receptors (MRs) located in the cytoplasm of target cells. The inactive MR complex is bound to heat shock protein 90 (HSP90), a protective chaperon protein, and is incapable of binding to target DNA sequences. Upon binding of aldosterone, HSP90 dissociates from the receptor–hormone complex, allowing movement of the activated receptor into the nucleus. The complex binds to target sequences of DNA referred to as mineralocorticoid-response elements (also termed hormone-responsive elements) that regulate transcription of downstream, mineralocorticoid-responsive genes. Protein products of these responsive genes, aldosterone-induced proteins (AIPs), cause Na^+ reabsorption and increase excretion of K^+ and H^+ in the late distal tubule and collecting duct. AIPs are thought to have multiple effects, including activation, redistribution, and de novo synthesis of Na^+ channels and Na^+/K^+-ATPase, changes in permeability of tight junctions, and increased mitochondrial production of ATP. These effects combine to cause an increase in Na^+ conductance of the luminal membrane and increased Na^+ pump activity in the basolateral membrane. As a result, Na and Cl transport is increased across tubular epithelial cells, and the lumen-negative transepithelial voltage is increased. Secretion of K^+ and H^+ into the tubular lumen increases with increasing voltages.

Aldosterone antagonists act by binding to the MR and facilitating the release of HSP90 from the steroid-binding subunit of the receptor. The unprotected MR complex is thought to be inactivated by proteases. In the absence of activated MRs, gene transcription is not induced, AIPs are not produced, and the physiological effects of aldosterone are blocked.

In addition to antagonism of aldosterone, spironolactone is thought to act in a manner similar to calcium channel blockers to cause direct vasodilation. By binding to plasma membrane sites, spironolactone may inhibit inward slow calcium channels and depress contractions dependent on release of calcium from the sarcoplasmic reticulum. Aldosterone antagonists have also been shown to increase nitric oxide vasodilation and circulating levels of atrial natriuretic peptide as evaluated in the dog. Spironolactone may produce antiarrhythmic effects by blocking the ether-a-go-go protein on the cardiac potassium ion channels (Gómez et al., 2005). Hence direct and aldosterone-mediated effects of the drug may contribute to its usefulness in treatment of cardiac disease (Endou and Hosoyamada, 1995).

Absorption and Elimination

In humans, spironolactone is absorbed moderately well (60–90%), is highly protein-bound, and is extensively biotransformed in the liver, exhibiting a first-pass effect. An active metabolite, canrenone, has a longer half-life than the parent drug and extends the biological effects of spironolactone to about 16 hours in humans. Spironolactone has approximately 60% oral bioavailability in dogs (Karim et al., 1976) and exhibits near proportional drug exposure (canrenone) from 0.7 to 8 mg/kg PO (Guyonnet et al., 2010). Peak diuresis occurs as late as 2–3 days after initiation of therapy. Aldosterone antagonists do not require secretion into the renal tubule to induce diuresis. Greater effects of aldosterone antagonists are expected as the concentration of aldosterone increases and conversely if low concentrations of aldosterone are present, less effects are expected.

Toxicity, Adverse Effects, Contraindications, and Drug Interactions

Hyperkalemia, dehydration, and hyponatremia are the most common side effects of aldosterone antagonists. When used alone, these drugs can also cause hyperchloremic metabolic acidosis. In humans, sexual side effects limit the use of spironolactone in some patients due to effects on progesterone and androgen receptors. This lack of receptor specificity drives continued efforts to identify a more-MR-specific antagonist for use in human medicine.

As previously noted, combination of any K^+-sparing diuretic, including spironolactone, with ACE inhibitors must be accomplished cautiously to avoid hyperkalemia. This is a clinically significant scenario that merits patient monitoring of plasma K^+ concentrations. Spironolactone may decrease oral bioavailability of digoxin due to increased p-glycoprotein expression (Ghanem et al., 2006), but may decrease digoxin renal clearance; therefore, therapeutic drug monitoring of digoxin is recommended when administered concurrently with spironolactone (O'Brien et al., 1985). The presence of spironolactone in plasma may also confound therapeutic drug monitoring of digoxin if a cross-reactive antidigoxin antibody is used in the assay. Aspirin apparently blocks spironolactone-induced natriuresis (Endou and Hosoyamada, 1995).

Therapeutic Uses

The effectiveness of aldosterone antagonists in promoting diuresis is largely dependent upon elevated concentrations of endogenous aldosterone. Aldosterone secretion increases upon activation of the renin–angiotensin–aldosterone system, which, in turn, responds to reductions in serum sodium, effective blood volume, and cardiac output, and decreases in serum K^+. Secondary hyperaldosteronism and edema are associated with cardiac failure, hepatic cirrhosis, nephrotic syndrome, and severe ascites. Spironolactone is used in veterinary medicine at a dose of 2–4 mg/kg/day orally in management of refractory edema associated with these conditions and has been used in the management of hepatic cirrhosis. In both human and veterinary medicine, aldosterone antagonists are commonly administered with a thiazide or loop diuretic to increase peak diuresis and to spare K^+.

Elevated aldosterone levels have been shown to be a useful prognostic indicator in heart failure, with higher levels correlated with a poorer prognosis. Activation of the renin–angiotensin–aldosterone system in arterial hypertension is thought to lead to remodeling of the myocardial collagen network with progressive cardiac interstitial fibrosis. As fibrosis increases, diastolic function deteriorates and pathological cardiac hypertrophy occurs. When aldosterone-mediated effects are blocked by spironolactone, progression of myocardial failure is presumably slowed. A clinical study in humans supported this contention by showing significant delays in progression of CHF in patients treated with spironolactone (Pitt et al., 1999). No comprehensive study has been published to verify this effect in veterinary patients. Diuretic efficacy of spironolactone was not demonstrated in a study combining the drug with furosemide in greyhound dogs (Riordan and Estrada, 2005).

A newer drug, eplerenone, reduced mortality in a human study of heart failure due to myocardial infarction (Weir and McMurray, 2005), and the drug has also been shown to have cardioprotective effects in animal models of myocardial failure (McMahon, 2003). Despite the potential side effect of hyperkalemia associated with coadministration of aldosterone-antagonists and ACE inhibitors, this combination with appropriate dosages has been deemed effective in management of CHF. Addition of furosemide, which enhances elimination of K^+, may decrease the risk of hyperkalemia. Patient monitoring for K^+ derangements is critical to safe implementation of this approach. In veterinary medicine, spironolactone may be useful in patients with CHF secondary to chronic valvular heart disease or dilated cardiomyopathy that become unresponsive to therapy with ACE inhibitors, digoxin, and furosemide.

Methylxanthines

Overview

Methylxanthines (aminophylline and theophylline) are primarily used for bronchodilator effects and are discussed in more detail in Chapter 48. Methylxanthines are adenosine receptor antagonists at lower dosages and higher dosage produce phosphodiesterase inhibitor effects. They produce weak diuresis and natriuresis. The mechanism of diuresis appears in part due to vasodilation of the renal afferent arterial increasing glomerular filtration rate. However adenosine receptors in the proximal convoluted tubule stimulate fluid and bicarbonate transport as well as Na^+/glucose and Na^+/phosphate symport (Rieg et al., 2005). Although not a primary choice as a diuretic, low-dose aminophylline has demonstrated efficacy as an adjunct in human pediatric patients that are refractory to furosemide diuresis (2005; 2012). The clinical utility in veterinary medicine has not been reported, but use of aminophylline as a diuretic adjunct in furosemide-resistant patients is an area that requires further research prior to recommended use in animals.

New and Experimental Agents

Aquaretics

Vasopressin (or arginine vasopressin, AVP) regulates water and solute excretion in the kidney by binding to V2 receptors in the principal cells of the renal collecting duct system. As one of three G-protein-coupled AVP receptor subtypes (V1a, V1b, V2), V2 receptors mediate the antidiuretic effects of AVP. V2 receptor antagonists, so-called aquaretic agents, promote solute-free water excretion. These antagonists hold considerable promise for treatment of edematous states associated with heart failure, liver cirrhosis, nephrotic syndrome, and syndrome of inappropriate secretion of antidiuretic hormone (Verbalis, 2006). While peptide derivatives of AVP have been found to have intrinsic antidiuretic properties, several nonpeptide antagonists are currently approved, including conivaptan and tolvaptan. These drugs typically increase urine volume and decrease urine osmolality and body weight without affecting urinary sodium excretion (Orita and Nakahama, 1998; Serradeil-Le Gal, 1998; Palm et al., 2006). Tolvaptan administered to healthy dogs dose dependently (0.3–10 mg/kg PO) increased urine volume and decreased urine osmolality, without increasing urinary sodium excretion (Miyazaki et al., 2007). Similar effects were observed in dogs with experimentally induced heart failure. In the healthy and CHF-induced experimental dogs, tolvaptan did not increase sympathetic or renin–angiotensin–aldosterone systems, but did increase AVP concentrations. No effects were observed on glomerular filtration rates, renal blood flow, or peripheral vascular resistance. No published clinical trials of tolvaptan in naturally occurring cardiac disease in dogs or cats are available. Conivaptan was shown to improve impaired cardiovascular parameters induced by intravenous infusion of AVP in a dog model, suggesting possible clinical usefulness (Yatsu et al., 2002).

Although vasopressin antagonists represent a promising area of drug development for induction of aquaresis, drugs that interfere with secretion of AVP from the neurohypophysis and drugs that directly inhibit water channels in the collecting ducts are also of interest. Aquaporin-CD, the water channel of the principal cell of the cortical and medullary collecting duct, has been cloned and provides an attractive site for drugs intended to inhibit diuresis.

Neutral Endopeptidase Inhibitors

Neutral endopeptidase (NEP) is an enzyme that rapidly degrades atrial natriuretic factor, the cardiac peptide hormone that increases sodium and water excretion, inhibits the renin–angiotensin–aldosterone system, and produces vasodilation. NEP inhibitors have been investigated for their possible usefulness in increasing circulating concentrations of atrial natriuretic factor and therefore increasing the fractional excretion of sodium. An experimental NEP inhibitor, ecadotril, has been investigated in induced heart failure in dogs (Solter et al., 2000; Mishima et al., 2002) and been shown to attenuate progression of disease. NEP inhibitors have been shown in dogs to enhance the actions of furosemide and prevent the furosemide-induced activation of the renin–angiotensin–aldosterone system (Kittleson and Kienle, 2007).

Dopamine Receptor Agonists

Dopaminergic receptor 1 (DA_1) and dopaminergic receptor 2 (DA_2) have both been considered targets for low–dose dopamine therapy of low output renal failure in dogs and humans. Efficacy of low-dose dopamine has been questioned and concerns have been raised over renal morbidity and gastrointestinal side effects. It has been noted that although dopamine increases renal perfusion and urine output, increases in creatinine clearance are not sufficient to achieve therapeutic efficacy in management of renal failure. In comparison, DA_1 selective agonists may be more effective at increasing renal blood flow, inducing diuresis and natriuresis, and increasing glomerular filtration rate. This is particularly true in cats, a species that does not respond well to the renal effects of dopamine. Fenoldopam, a selective DA_1 agonist, has been investigated in both dogs and cats. In cats, fenoldopam at a dose of 0.5 μg/kg/min induced diuresis in a delayed manner, by 6 hours postadministration, increased urine output, sodium excretion, fractional clearance of sodium, and elimination of creatinine (Simmons et al., 2006).

Angiotensin Converting Enzyme Inhibitors and Angiotensin Receptor Antagonists

Chemistry/Formulations

Numerous angiotensin converting enzyme (ACE) inhibitors are approved for use in humans and animals. Captopril was the first ACE inhibitor marketed and is available in oral dosage from 12.5 to 100 mg tablets and in combination with hydrochlorothiazide. Enalapril maleate is a prodrug ester approved for use in dogs and in humans and is available in tablets ranging from 1 to 20 mg and as a 1 mg/mL oral solution. Enalaprilat is the active drug and is available as an injection (1.25 mg/mL), but rarely used in veterinary medicine. Benazepril hydrochloride is available as 5–40 mg tablets and is approved in some countries for use in dogs and cats. Imidapril and ramipril are also approved for use in some countries for dogs. Although numerous other ACE inhibitors are available, they are less commonly used.

Angiotensin receptor antagonists, also known as angiotensin receptor blockers (ARBs) are approved for use in humans. Losartan, telmisartan, valsartan, and irbesartan, available for oral administration, have rarely been used in dogs and cats, but may see increasing use in veterinary medicine.

Mechanisms and Sites of Action

The action of ACE inhibitors is to decrease the metabolism of angiotensin I (ATI) to angiotensin II (ATII), which produces profound physiological effects, as described in Section Renin–Angiotensin–Aldosterone System (Figure 24.10). ACE also inactivates bradykinin, therefore ACE inhibitors increase bradykinin and subsequently prostaglandins both of which may provide additional benefits. The efficacy of the different ACE inhibitors are similar in humans, but differences in pharmacokinetics and individual sensitivity to adverse effects are the primary differences for ACE inhibitor selection for specific patients.

Angiotensin receptor blockers are selective antagonists of the angiotensin receptor type 1 (AT_1). Although they are considered reversible antagonists, their (or their active metabolite) antagonism cannot be overcome with higher concentrations of ATII. The ARBs are specific for inhibiting ATII in contrast to ACE inhibitors that can additional effects such as decreased bradykinin metabolism.

The ACE inhibitors decrease ATII formation while the ARBs directly inhibit ATII binding to AT receptors, decreasing ATII effects including direct vasoconstriction, rapid and slow pressor responses, vasopressin and aldosterone release, sympathetic nervous system tone, enhanced release of norepinephrine, and adrenal catecholamine release. Although ACE inhibitors and ARBs are expected to have minimal blood pressure effects in healthy animals, animals with high concentrations of renin and ATII can have profound effects. In humans, ACE inhibitors and ARBs are usually started at a low dose and increased over time to minimize acute hypotension. However, hypotension is much less of a problem in dogs and cats as, in general, the ACE inhibitors and ARBs are relatively low-efficacy vasodilatory drugs.

Absorption and Elimination

Most ACE inhibitors are formulated as a prodrugs to enhance oral bioavailability. The exception is captopril, which is an active drug. The oral bioavailability of most ACE inhibitors in dogs and cats are low, but sufficient for clinically relevant effects. In contrast, the oral bioavailability of enalapril in horses is very low and enalapril is not an effective PO drug in horses (Gardner et al., 2004). Additionally, when benazepril, ramipril, quinapril, and perindopril were administered PO to horses only benazepril produced reasonable effects in horses (Afonso et al., 2013). The oral bioavailability of enalapril and benazepril are not affected by feeding, but the oral bioavailability of captopril, imidapril, and ramipril are decreased by administration with food. Enalapril, benazepril, imidapril, and ramipril are prodrugs metabolized to the active metabolites enalaprilat, benazeprilat, imidaprilat, and ramiprilat. Enalaprilat and captopril are primarily eliminated unchanged in the urine while benazeprilat, ramiprilat, and imidaprilat are approximately equally eliminated in the urine and bile. Therefore animals with decreased renal function will have decreased elimination of captopril and enalaprilat, which could lead to exaggerated and adverse effects if dosages are not adjusted. Thus benazepril, ramipril, and imiapril are preferred in animals with decreased renal function. The doses of ACE inhibitors are: enalapril 0.25–0.5 mg/kg PO q 12–24 h; benazepril 0.25–0.5 mg/kg PO q 12–24 h; imidapril 0.25 mg/kg PO q 24 h; and ramipril 0.125–0.25 mg/kg PO q 24 h.

The pharmacokinetics of the oral ARBs losartan, telmisartan, valsartan, and irbesartan have been reported in dogs, but not in cats (Huang et al., 2005; Baek et al., 2013). In cats, telmisartan produced greater and longer lasting effects than losartan and irbesartan. However, limited dosage ranges were assessed and the pharmacokinetics, including oral bioavailability and half-life, are unknown; therefore, it is unclear if telmisartan is a better choice in cats or if the dosages of losartan and irbesartan were inappropriate (Jenkins et ., 2015). The oral bioavailability of losartan in dogs is relatively low, 23–33%, but the absolute oral bioavailability of the other ARBs have not been reported (Christ et ., 1994). In humans, the oral bioavailability of most ARBs is <50%. In humans, losartan is metabolized to an active metabolite, E-3174, which is more potent than losartan, but minimal amounts of the metabolite are produced in dogs (Suzuki et al., 2001). Metabolism of losartan has not been reported in cats. Despite the lack of the active metabolite in dogs, effects of losartan against exogenous ATII were profound, but of short duration. In humans, ARBs are primarily eliminated by hepatic metabolism and biliary secretion with changes in renal function having minimal effects on their pharmacokinetics. Food markedly decreases the oral absorption of telmisartan and valsartan but not losartan and irbesartan in humans. The effect of food on the pharmacokinetics of ARBs in dogs has not been extensively evaluated, therefore it would be prudent to administer to fasted animals until further data are available.

Toxicity, Adverse Effects, Contraindications, and Drug Interactions

Angiotensin converting enzyme inhibitors in dogs are cats are, overall, well tolerated. Acute renal failure is an adverse effect with ACE inhibitors and ARBs that warrant close monitoring. Renal adverse effects may be more severe and rapidly progressive in animals treated with enalapril or captopril as drug accumulation occurs once renal dysfunction starts, but renal adverse effects can occur with any ACE inhibitor. Renal failure risks are increased in animals with renal hypotension as ATII vasoconstriction of the efferent arteriole maintains renal blood pressure, but is blocked by ACE inhibitors and ARBs. In contrast, animals with renal hypertension and proteinuria can have therapeutic benefits of ACE inhibitors and ARBs. Animals that are dehydrated and hyponatremic are at higher risk for renal failure. Serum creatinine should be routinely monitored for development or worsening of azotemia, initially within a week of initiating therapy and then periodically throughout therapy. If azotemia is detected, decreasing the dose of concurrent diuretics (decreasing dehydration and hyponatremia) may be appropriate in addition to decreasing or discontinuing ACE inhibitor or ARB therapy.

Hyperkalemia due to ACE inhibitors and ARBs in animals is rare unless combined with potassium-sparing diuretics or renal failure. The RAAS is important for the development of normal kidney anatomy in utero and exposure to ACE inhibitors and ARBs result in numerous birth defects and should be avoided in pregnant animals and neonates. Although hypotension can occur with ACE inhibitors and ARBs, it is uncommon in animals unless the animal is dehydrated or receiving other vasodilators. In humans, coughing and angioedema is a common adverse effect due to enhanced bradykinin from ACE inhibitors, but not ARBs; however, coughing and angioedema are rarely or not reported adverse effect in animals.

There is the potential for drug interactions with ACE inhibitors and ARBs in dogs and cats. The risk of renal adverse effects appears to increase and the antihypertensive effects diminished when combined with NSAIDs that affect prostaglandin formation (KuKanich et al., 2012). Concurrent administration of vasodilators with ACE inhibitors are expected to produce exaggerated vasodilatory effects, which could result in systemic hypotension in addition to renal hypotension, increasing the risk of renal toxicity. The concurrent use of diuretics can lead to hyponatremia and dehydration, which increases the risk of renal toxicity. Likewise, the concurrent use of nephrotoxic drugs, including but not limited to aminoglycosides, amphotericin B, and cisplatin, increases the risk of renal toxicity. Combination of ACE inhibitors with potassium sparing diuretics, including spironolactone, increases the risk of hyperkalemia. In humans, ACE inhibitors decrease the clearance of digoxin, but this interaction has not been reported in animals.

Therapeutic Uses

Angiotensin converting enzyme inhibitors are commonly administered to dogs with chronic heart disease. Although ACE inhibitors are used in cats with hypertrophic cardiomyopathy, there are less data available demonstrating therapeutic benefits. However, numerous laboratory studies and clinical trials are available in dogs demonstrating the benefit of ACE inhibitors in dogs with chronic heart disease (dilated cardiomyopathy and chronic valvular diseases) often combined with other therapeutics, including diuretics (primarily furosemide and spironolactone), digoxin, and pimobendan (Chapter 21), as appropriate (Lefebvre et al., 2007). The most important clinical effects for clients are improved quality of life and increased survival times when affected dogs with symptomatic heart disease are treated with ACE inhibitors in combination with appropriate concurrent therapeutics. Dogs treated chronically had greater decreases in pulmonary edema, respiratory effort and coughing, and improved appetite, exercise tolerance/ mobility/ activity, attitude, and general demeanor with the addition of ACE inhibitors. Despite the clinical improvement, specific physiological measurements such as cardiac output, stroke volume, pulmonary arterial pressures, and systemic vascular resistant are often unaffected. The data are more equivocal with dogs exhibiting asymptomatic/ subclinical heart disease and routine use of ACE inhibitors in these dogs are not currently recommended. There are no studies reporting the efficacy of ARBs in dogs or cats with naturally occurring chronic heart disease, but the availability of cost-effective ARBs will likely increase their use in veterinary medicine.

ACE inhibitors are also administered to dogs and cats for the treatment of proteinuria and glomerular diseases (Brown et al., 2013; King et al., 2006). Dogs with a urine protein to creatinine ratio (UPC) exceeding 0.5 are recommended to be treated with an ACE inhibitor. Clinical trials have demonstrated efficacy of enalapril in decreasing proteinuria in dogs with glomerular disease and delayed the onset of azotemia (Brown et al., 2013). Although studies demonstrating the efficacy of benazepril on dogs with proteinuria are lacking, benazepril is often administered to dogs with elevated UPC and glomerular disease. An advantage of benazepril is that its pharmacokinetics are minimally affected by renal dysfunction, compared to enalapril which is significantly altered. There are no reports of ARBs used for the management of naturally occurring proteinuria and glomerular disease in dogs, but with the availability of cost-effective ARBs they will likely be used more commonly. In cats, benazepril has been recommended for the management of chronic kidney disease (CKD) with elevated UPC (King et al., 2006). Benazepril significantly decreased UPC and maintained plasma protein concentrations compared to placebo in cats. However, there was no significant difference in survival time and need for parenteral fluids/ euthanasia/ death due to renal failure, but there was large variability. Similarly, telmisartan significantly decreased UPC in cats with CKD (Sent et al., 2015).

ACE inhibitors as a sole treatment for hypertension in dogs and cats have a low efficacy and most often do not provide a clinically desired effect. Vasodilators such as amlodipine (Chapter 21) are often administered concurrently with ACE inhibitors to achieve clinically desired decreases in blood pressure.

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Section 7

Drugs Acting on Blood and Blood Elements

25

Anticoagulant, Antiplatelet, and Hemostatic Drugs

Mark G. Papich

There are only a small number of drugs used in veterinary medicine to affect the coagulation system. Although important coagulopathies have been identified in animals, and thromboembolism is an important component of several diseases, we lack well-controlled studies to identify the best drug to manage these problems. We often use drugs that are approved for humans to manage thromboembolic diseases but the doses and frequency of administration of these drugs in animals often is undetermined and we extrapolate doses from human medicine, or use anecdotal information to base therapy. For example, there is not a clear understanding for the most effective antiplatelet dose of aspirin to administer to dogs and cats, or whether it is even effective. Likewise, although heparin is an important anticoagulant – including the low-molecular-weight forms – the optimum dose is unclear.

The conventional thinking regarding the use of anticoagulant and antiplatelet drugs is that coagulation disorders arise either on the arterial side or the venous side of the circulation. Disorders of high shear force and arterial injury induce platelets to become activated and induce a hypercoagulable state. The resulting clot is platelet rich, with platelets embedded in fibrin (the so-called *white thrombi*). Coagulation disorders arising on the venous side are often attributed to slow or sluggish blood and blood pooling. These clotting disorders are composed predominantly of trapped red blood cells embedded in fibrin (the so-called *red thrombi*). These clots can produce pulmonary thromboembolism. Therefore, treatment of clotting disorders on the venous side traditionally employ anticoagulant drugs and clotting disorders on the arterial side employ antiplatelet drugs. Obviously, the clotting disorders cannot so easily be divided into these two categories and for some diseases both components are important. For example, one of the most serious diseases for which thromboembolism plays a role is heart disease in which blood may pool in the atrium and send emboli to the pulmonary circulation, resulting in pulmonary thromboembolism. These occur in the pulmonary arteries and both antiplatelet and anticoagulant drugs have been used to prevent these problems. Dogs with immune-mediated hemolytic anemia (IMHA) have generalized thromboembolic disease of both veins and arteries and pulmonary thromboembolism (PTE) is a common complication (Kidd and Mackman, 2013). Dogs with IMHA may be at more risk because some of the drugs used to treat this disease, such as glucocorticoids and cyclosporine, may increase platelet activity (Thomason et al., 2016; Kidd and Mackman, 2013)., Evidence-based recommendations for other diseases in which thromboembolism plays a role in dogs and cats are found in the review by Lunsford and Mackin (2007).

Drugs such as heparin and coumarin inhibit hemostasis by impeding clot formation. Both are used for treatment and prevention of thromboembolic disorders in animals. Newer anticoagulants – factor Xa inhibitors (rivaroxaban, apixaban) and thrombin inhibitors (dabigatran) – are becoming more popular in human medicine but their use in animals is still rare. Antiplatelet drugs in veterinary medicine are limited to aspirin and clopidogrel. We have good evidence for effectiveness of clopidogrel, but the value of aspirin as an antiplatelet agent is being questioned. There are also hemostatic agents – those intended to increase blood clotting, or fibrin formation – but these have little use in clinical veterinary medicine and will be covered only briefly in this chapter.

Hemostasis

Hemostasis refers to prevention or control of hemorrhage. Physiological control systems operate to ensure fluidity of blood under normal conditions, yet opposing systems promote coagulation when the circulatory system is invaded (Edelberg et al., 2001). Hemostasis is achieved through a series of interdependent mechanisms, including vascular spasm of the injured artery or

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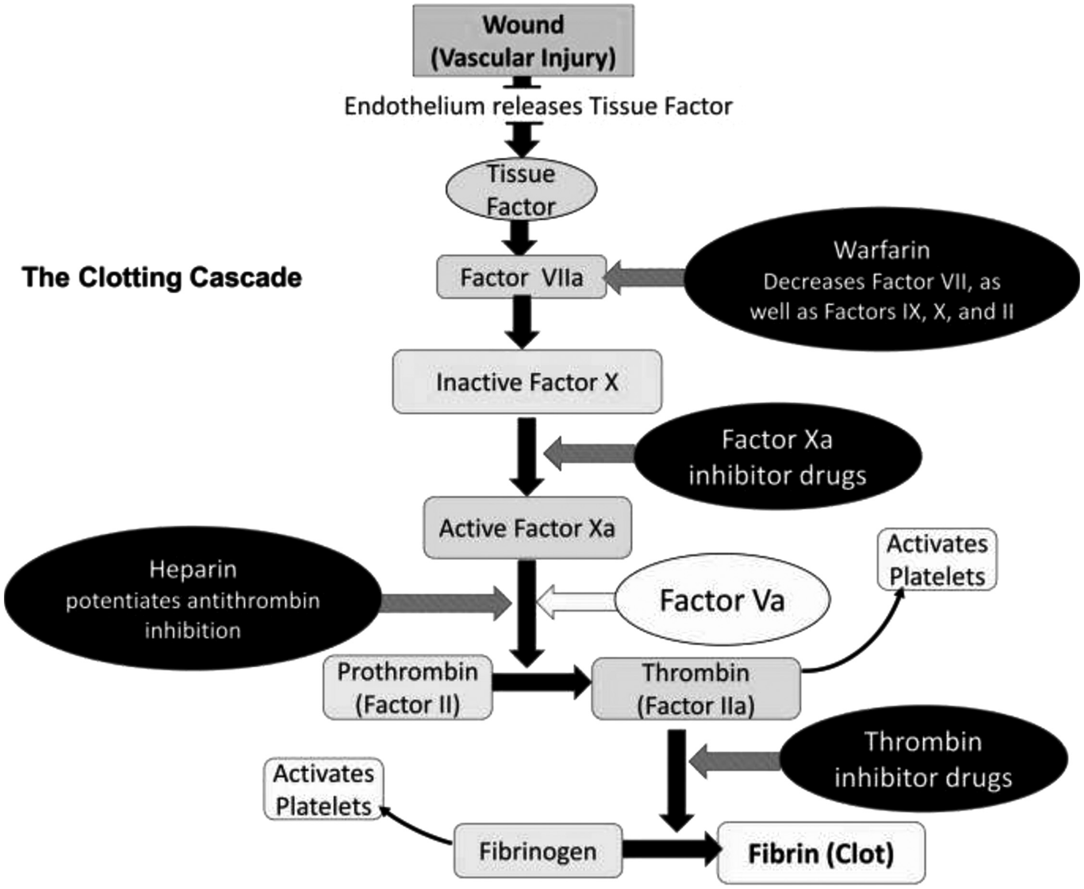


Figure 25.1 The sequence of events that lead to fibrin formation and clotting in response to vascular injury. Sites of drug action are shown in black ovals. See text for discussion of drugs in each group.

vein, local aggregation of platelets into a plug, coagulation of the blood into a clot, and subsequent dissolution of the formed clot by fibrinolysis (Figure 25.1 and Figure 25.2). The basic process of hemostasis can be separated into the vascular, platelet, coagulation, and fibrinolysis phases. The phases overlap considerably, and events in one step promote and even cause development of subsequent phases.

Vascular and Platelet Phases

The vascular and platelet phases are closely allied, with the vascular endothelium having a multitude of both anticoagulant and procoagulant functions. Ordinarily, platelets circulate in an inactive state. Nitric oxide (NO) and prostacyclin (PGI_2) released by vascular endothelial cells help to maintain the inactive state of platelets. Reduced levels of NO may contribute to thromboembolic disease in some animals. The endothelial cells also produce ADPase, that helps to degrade adenosine diphosphate (ADP). Adenosine diphosphate is a potent stimulator of platelet activation. Immediately after a blood vessel is injured and the subendothelium exposed, tissue factor (TF) and von Willebrand factor (vWF) are exposed, which attracts platelets to the injured endothelium. Platelets have specialized receptors for vWF and TF that facilitate this attraction to the endothelium. For platelets to properly attach to a traumatized area, vWF must be present because the platelets express a vWF integrin receptor that facilitates adherence. Animals deficient in vWF may have bleeding disorders that may be treated by medications that stimulate vWF. Agents such as desmopressin acetate (discussed in Section Desmopressin Acetate) are used for this role. Activation of platelets, in turn, initiates the coagulation factor cascade (Figure 25.1). Once adhesion has occurred, the platelets undergo a change in shape and release diverse substances that recruit further platelets to the clot and further promote the coagulation cascade. The substances involved in activation include ADP, adenosine triphosphate (ATP), serotonin (5-HT), platelet factor 3 and 4, thromboxane A_2 (TXA_2), and platelet-derived growth factor. This aggregation yields a rather loosely formed plug or platelet thrombus at the injury site. The smooth muscle of the vascular wall contracts and platelets adhere to the injured site (Figure 25.2). The local vasoconstrictor response, or

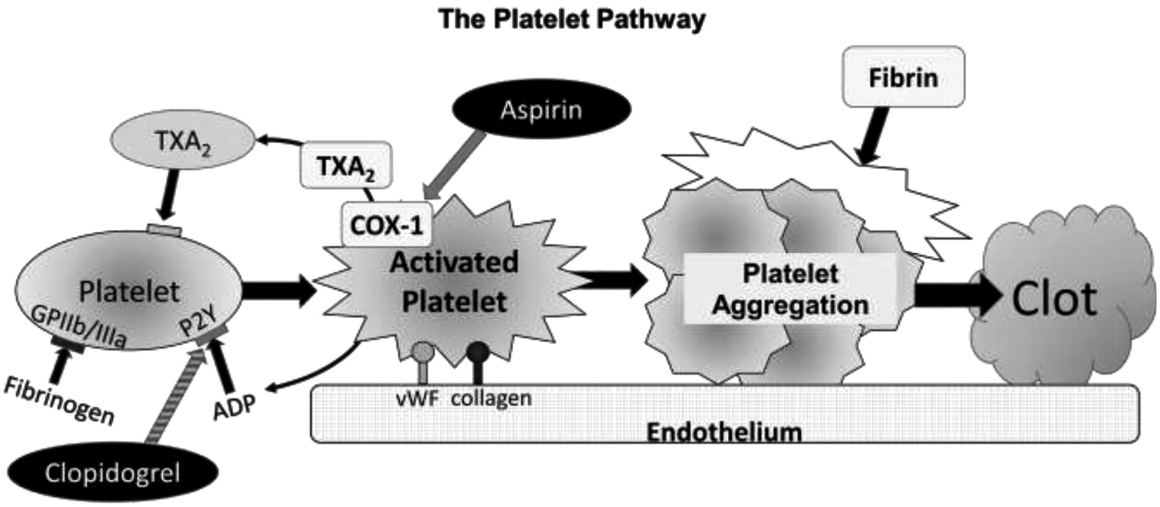


Figure 25.2 The sequence of events, simplified here, to show how activated platelets lead to platelet incorporation into fibrin clot. Sites of drug action are shown in black ovals. Note that the source of TXA$_2$ and ADP are from activated platelets, in which they amplify platelet aggregation by secreting platelet-activating substances. See text for discussion of drugs in each group. ADP, adenosine diphosphate; COX, cyclooxygenase; TXA, thromboxane.

vascular spasm, mechanically retards the flow of blood escaping from the vessel. Vascular spasm may be partly a local reflex or myogenic response and partly humoral, owing to vasoactive agents released from platelets and nearby endothelial cells. Local vasoconstriction lasts as long as 20–30 minutes, during which the ensuing phases of platelet aggregation and blood coagulation take place.

Involvement of Prostaglandins

Adenosine diphosphate is a potent chemical activator of platelet aggregation, which in turn activates a phospholipase that acts on membrane phospholipid to yield arachidonic acid. The latter is transformed by a platelet cyclooxygenase into short-lived but potent aggregating compounds called cyclic endoperoxides (prostaglandins [PGs] G_2 and H_2). (These compounds are also discussed in Chapter 20.) These endoperoxides are converted by cyclooxygenase-1 (COX-1, platelet thromboxane synthase) to the potent aggregating compound called TXA$_2$ (Figure 25.2). Thus platelet clumping initiates formation of chemical agents that promote further platelet aggregation. In contrast, prostacyclin (PGI$_2$) formed by COX-2 is a PG that inhibits platelet aggregation and acts as a counterbalance to thromboxane. Prostacyclin is formed from arachidonic acid and intermediate cyclic endoperoxides. Prostacyclin synthase (COX-2), the enzyme responsible for transformation of PGI$_2$ from the cyclic endoperoxides, is localized in the vascular endothelium rather than in platelets. Once the platelets adhere and aggregate, the contractile proteins in the platelets contract and the platelet plug becomes more tightly bound and organized by incorporation of fibrin strands formed during coagulation (Figures 25.1 and 25.2).

Coagulation Phase

The clotting cascade is comprised of a sequence of events primarily involving a series of activation of *zymogens.* The zymogens are precursor proteins that are converted to an active protease by cleavage of peptide bonds in the precursor molecule. Once activated, these zymogen coagulation factors activate other factors in a sequence until the final protease, thrombin, is generated (Figure 25.1). The clotting factors are designated by Roman numerals (II, VII, VIII, IX, X, etc.; Table 25.1) and once activated,

Table 25.1 Blood coagulation factors and their common names

Factor	Common name	Drug inhibitor
Factor I	Fibrinogen	
Factor II	Prothrombin	Heparin
Factor III	Tissue thromboplastin	
Factor IV	Calcium	Calcium chelators (e.g., EDTA used in test tubes).
Factor V	Proaccelerin	
Factor VII	Proconvertin; serum prothrombin conversion accelerator	Warfarin
Factor VIII	Antihemophilic factor	
Factor IX	Plasma thromboplastin component; Christmas factor	Warfarin
Factor X	Stuart–Prower factor	Heparin, rivaroxaban
Factor XI	Plasma thromboplastin antecedent	
Factor XII	Hageman factor	
Factor XIII	Fibrin stabilizing factor	

are shown with the "a" subscript (IIa, VIIa, Xa, etc.). Calcium also is important in these reactions. Calcium ions (Ca^{++}), referred to as clotting factor IV (Table 25.1), are required for all coagulation reactions except the first two steps in the intrinsic pathway. This dependency is used in the laboratory; Ca^{++}-complexing agents such as citrate and EDTA are used as in vitro anticoagulants (discussed in Section In Vitro Anticoagulants and Clotting Tests).

Fibrinolysis Phase

A plasma β globulin called plasminogen is bound to fibrin and incorporated into the clot along with other plasma constituents. Fibrinolysis is initiated when plasminogen is activated by local agents to plasmin. Tissue-type plasminogen activator is produced by endothelial cells and fibroblasts and utilizes fibrin as a cofactor in the conversion of plasminogen to plasmin. Plasmin is a proteolytic enzyme that digests the fibrin chains into soluble polypeptides, thereby preventing further fibrin polymerization. Plasmin also digests other substances in the clot and surrounding blood, for example prothrombin, fibrinogen, and clotting factors V, VIII, and XII. Formation of plasmin results in dissolution of the clot and also in hypocoagulability of the blood because of loss of clotting factors. Thus fibrinolysis represents the physiological converse of the coagulation process. It serves as a defense mechanism against overactivity of the coagulation mechanism.

Natural Anticoagulants

Antithrombin (AT, formerly called antithrombin-III) is an endogenous anticoagulant. It is a globulin with a molecular weight of 65,000 and has the ability to inactivate factors Xa, IXa, XIa, and XIIa, and thrombin. The anticoagulant activity of heparin is due to its interaction with AT. When AT binds to heparin it markedly increases the rate of inactivation of factor Xa and thrombin. Antithrombin also is called the heparin cofactor. This enzyme is an important thrombin antagonist, but it additionally inhibits activated forms of factors IX, X, XI, and XII. Antithrombin combines in a stable manner with the enzymatically active binding sites of these factors, thereby preventing their accessibility to subsequent substrates in the clotting cascade.

In Vitro Anticoagulants and Clotting Tests

Two categories of chemicals are used to prevent coagulation of harvested blood: those employed in samples of blood intended for physical or chemical examination and those employed to preserve blood for transfusion. Some of these agents can be used to prevent clotting both in vitro and in vivo (heparin), while others are best used only in vitro because of their toxicity (oxalates).

Typically, blood clots in 4–8 minutes when added to a glass tube (red-top tube). To prevent clotting, anticoagulants may be added for blood sample diagnostic tests. The chelator ethylenediaminetetra acetic acid (EDTA*)* can be added to tubes to chelate calcium and prevent clotting (purple-top tube). Citric acid also can be added to bind calcium. Calcium is an important cofactor for many of the clotting factors. If calcium is then added to a decalcified tube, clotting resumes in 2–3 minutes. If negatively charged phospholipids and a substance such as aluminum silicate (kaolin) is added, the clotting is shorted to less than 1 minute. This is known as the *activated partial thromboplastin time* (aPTT). Another test of coagulation is the *prothrombin time* (PT), which is performed by adding calcium to a decalcified blood sample and then adding thromboplastin (tissue factor and phospholipids). These tests may be performed in the laboratory to detect clotting disorders and are discussed more in internal medicine and laboratory medicine textbooks. For monitoring warfarin treatment the *international normalization ratio* (INR) is recommended. This ratio is derived from a normalization of the PT clotting test. Prothrombin times are reported in seconds and recorded as a ratio of the prothrombin time of the patient to the mean normal prothrombin time of the laboratory and calculated as the international normalized ratio (INR). Because test reagents can differ, the INR is the most reliable way to monitor the prothrombin time (Hirsh, 1991). The PT measurement is converted to the INR using the following formula:

$$\mathrm{INR} = \left[\mathrm{PT}_{\mathrm{PATIENT}}/\mathrm{PT}_{\mathrm{REFERENCE}}\right]^{\mathrm{ISI}}$$

where the PT is the prothrombin time of the patient and reference (from pooled normal blood), and ISI is the international sensitivity index, which is supplied by the manufacturer of the test reagent and indicates the degree of sensitivity of the PT. (In most laboratories the value of ISI is 1.0, simplifying the calculation of this ratio.)

The "gold standard" test for heparin activity is the measure of the plasma factor Xa. This factor is inhibited by heparin and is used for monitoring and adjusting heparin administration to people (Vandiver and Vondracek, 2012). Based on studies in people the therapeutic range for anti-Xa activity is 0.35–0.70 units anti-Xa/ml (Brooks, 2004). Because the aPTT test can produce variable results, the direct factor Xa assay is considered more reliable for monitoring heparin (Brooks, 2004). Despite these advantages, at this time, the direct factor Xa assay is not routinely available in veterinary laboratories and impractical for clinical veterinary medicine (McLaughlin et al., 2017). Moreover, it is a chromogenic assay and can be influenced by other factors that impart color changes in the sample.

More recently, the thromboelastography (TEG) test has been used to diagnose hypo- and hypercoagulable states in animals (Wiinberg et al., 2008). This is a whole-blood assay that tests the viscoelastic properties of clots and provides an assessment of clot forming kinetics and clot strength. The test has become valuable in veterinary medicine to assess hemostasis and is available in many of the large veterinary hospitals.

Other anticoagulant agents used in laboratory examination of blood include sodium oxalate in a concentration of 20% at 0.01 ml/ml (2 mg/ml) blood, and sodium citrate in a concentration of 25% at 0.01 ml/ml (2.5 mg/ml) blood. EDTA (mentioned above in this section) can prevent coagulation when used in a concentration of 1 mg/5 ml blood. Heparin also can be used to prevent coagulation and can be added to a collection syringe (*washing the syringe with heparin)*, added to a collection tube (green tube) or by adding 75 units to each 10 ml whole blood.

Anticoagulants used for blood and blood component transfusions ideally should maintain the function of the individual components and have preservative effects. Such anticoagulant agents include:

1) Acid citrate dextrose (ACD solution), consisting of sodium citrate 25 g, citric acid 8 g, dextrose 24.5 g, and distilled water to make a total volume of 1000 ml, given at the level of 15 ml/100 ml blood. The toxicity of citrated blood injected intravenously varies with rate of injection and total dose. The lethal dose of sodium citrate for the dog is estimated to be about 132 mg/kg after extensive hemorrhage; in the normal intact dog the lethal dose is about 286 mg/kg.
2) Citrate-phosphate-dextrose-adenine (CPDA-1) is one of the most commonly used anticoagulants in human and veterinary transfusion medicine. It can maintain a high level of erythrocyte posttransfusion viability for up to 20 days in dogs (Price et al., 1988).

Heparin

Heparin (heparin calcium and heparin sodium) can be used in vivo and in vitro to inhibit clotting. Heparin has both antithrombotic and anticoagulation effects, which are not necessarily dependent on each other. Heparin is prepared from bovine lung tissue or porcine intestinal mucosa. Both calcium and sodium salts of heparin are available for therapeutic use and occur as a white hygroscopic powder that is easily soluble in water. It is composed of polymers of alternating derivatives of α-ᴅ-glucosamine (*N*-sulfated, *O*-sulfated, or *N*-acetylated) and uronic acid (α-ʟ-iduronic acid or β-ᴅ-glucuronic acid) joined by glycosidic linkages. Heparin used parenterally has a direct and almost instantaneous action on the coagulation process. By contrast, the coumarin derivatives (for oral administration) have an indirect anticoagulant effect by acting as vitamin K antagonists in the hepatic synthesis of select coagulation factors. Subsequently, action by the coumarin derivatives is delayed for several hours.

The medical use of heparin consists of the conventional form of heparin, also called unfractionated heparin (UFH), and the more recent use of low-molecular-weight heparin (LMWH), which is discussed in Section Low-Molecular-Weight Heparins. Unfractionated heparin has a molecular weight of 5,000–30,000 (average 15,000). Heparin sodium is the most common form of UFH. Heparin differs from LMWH by the anti-factor Xa/anti-factor IIa ratio. Heparin has a ratio of 1 : 1, but the LMWHs have ratios of 2 : 1 or higher (ratios are given for each product listed in this chapter).

The action of heparin is to potentiate the action of antithrombin (AT), discussed in Section Natural Anticoagulants. It produces a conformational change in the AT molecule that significantly enhances its inhibitory effect on various activated coagulation factors. The predominant effect of this binding is inhibition of thrombin (factor IIa), as well as factors IXa and Xa, by forming complexes. The action of heparin accelerates the activity of AT by approximately 1,000-fold. This difference in activity between UFH and LMWH is that conventional heparin (UFH) inactivates both factor Xa and thrombin, but LMWHs predominantly inactivate factor Xa because they are not large enough to bind thrombin and AT concurrently. The additional inactivation of thrombin by UFH increases the anticoagulant activity, but also increases the risk of bleeding.

The dose form of heparin is standardized by units rather than mg doses. The standardization is performed by bioassay. In 2009, the standardization underwent changes because of contamination problems in people in 2007–2008 that led to serious adverse events. The contamination problems have been rectified and unitage of heparin is now formulated to an international standard. The United States Pharmacopeia (USP) standard potency is not less than 180 USP heparin units in 1 mg of dried powder.

Indications and Clinical Uses

Heparin is administered to prevent and treat hypercoagulability disorders and prevent coagulation disorders such as thromboembolism, venous thrombosis, disseminated intravascular coagulopathy (DIC), and pulmonary thromboembolism. Use in specific situations in animals is primarily anecdotal or derived from the clinical experience in people. There are few outcome-based studies to evaluate efficacy to support specific guidelines for therapy in veterinary medicine.

Heparin is usually administered subcutaneously (SC) because intramuscular (IM) administration may create a hematoma. After many years of administering heparin to patients with thromboembolic disorders, veterinarians have still not determined the optimal dose or dose frequency. Clinical doses to prevent thrombosis in canine patients with immune-mediated hemolytic anemia (IMHA) has not been effective (50–300 units/kg, SC, q 6 h) but when doses were adjusted based on anti-Xa activity, they were more effective and doses in individual dogs were as high as 560 mg/kg q 6 h. Unfortunately, the anti-Xa assay used in that study is not routinely available in veterinary laboratories. Heparin is also used in horses to prevent thrombosis in patients at risk, but dosage regimens are based on extrapolation from other animals or people, or clinical opinion.

Heparin is definitely *not* a drug for which *one dose fits all.* A fixed dose for every patient will not produce consistent and reliable effects. As the study on treatment of IMHA (Helmond et al., 2010) demonstrated, heparin doses may vary tremendously among patients, depending on the severity of the underlying disease and circulating AT levels (Kidd and Mackman, 2013). A prospective study in dogs with IMHA showed that UFH administered at 300 units/kg SC every 6 hours was generally inadequate to attain the anti-Xa activity in a targeted range (Breuhl et al., 2009). Patients with low AT may not respond as well as those with adequate AT levels. Other variables that may affect the efficacy are variable pharmacokinetics among animals and protein binding. Some clinical syndromes result in low AT levels, which is important to the effectiveness of heparin. Ideally, dose adjustments should be tailored to individual patients by monitoring clotting times. The test most often available to veterinarians is the activated partial thromboplastin time (aPTT). Doses have been adjusted to maintain activated aPTT clotting times at 1.5 to 2.5 times normal. A measurement of anti-factor Xa activity is considered the “gold standard” for determining whether therapeutic UFH doses are attained in people. The target range for anti-Xa activity is 0.35–0.70 units/ml (based on recommendations from human medicine). The problem is that the ideal range for measuring activity in dogs, cats, and horses is not established, and availability of the anti-factor Xa test is not available in most veterinary laboratories. The conclusion in a review paper was that adjusting heparin therapy based on aPTT prolongation does not consistently correlate with anti-Xa activity in dogs (Kidd and Mackman, 2013). In healthy dogs a dose of 500 units/kg SC every 6 hours was not reliable and treatment should be monitored in each patient (Mischke et al., 2001). Because it has been difficult to derive scientifically based dosage regimens, the doses in animals are primarily based on anecdotal uses.

Heparin sodium is available in 1000- and 10,000-units/ml injection vials. For dogs and cats the most common dose used for low-dose prophylaxis is 70 units/kg q 8–12 h SC. For treatment of on-going coagulation problems the dose for dogs is 100–200 units/kg IV loading dose, followed by 100–300 units/kg q 6–8 h SC. The dose is adjusted by monitoring, which may require an increase to 500–600 units/kg if necessary. In more severe cases and high-risk patients, it is recommended to start with 500 units/kg SC as the initial dose, then administer 500 units/kg q 12 h with follow-up monitoring to adjust the dose. To administer a constant rate infusion, use a loading dose of 100 units/kg IV, followed by CRI 18 units/kg/h. The initial dose for cats is 300 units/kg SC, q 8 h. As with dogs, increase to 500 units/kg if necessary.

The typical dose recommended for horses – and used in other large animals – is an initial dose of 150 units/kg SC, followed by 125 units/kg SC q 8–12 h (Moore and Hinchcliff, 1994). These authors discuss the various clinical uses that include jugular vein thrombosis, laminitis, and complications from surgery. The pharmacokinetics in horses are nonlinear and the activity cannot be predicted from plasma heparin pharmacokinetics (McCann et al., 1995). Therefore, monitoring of aPTT is needed to individually adjust the dose. It is important to note that *the doses listed above have not been tested in clinical studies*; they are extrapolated from human medicine or based on recommendations from veterinary specialists.

Adverse Effects

Adverse effects are caused by excessive inhibition of coagulation and produce bleeding problems in patients. Heparin-induced thrombocytopenia, a problem in people, has not been cited as a problem in animals. If excessive anticoagulation and bleeding occur as a result of an overdose, protamine sulfate should be administered to reverse heparin therapy (discussed in Section Reversing Agent for Heparin: Protamine Sulfate). It should not be used in animals unless there is an ability to monitor in order to prevent life-threatening bleeding.

Low-Molecular-Weight Heparins

Low-molecular-weight heparins (LMWH) used in veterinary medicine include tinzaparin (Innohep®), enoxaparin (Lovenox®), and dalteparin (Fragmin®). Most of the veterinary experience has been with dalteparin and enoxaparin. As discussed below, these drugs have several advantages in people over the conventional UFH (Weitz, 1997). These advantages include greater safety, as well as more favorable and predictable pharmacokinetics. However, as described in Section Indications and Clinical Uses, these advantages have not been established for the veterinary uses.

Dalteparin

Dalteparin (Fragmin) is characterized by a molecular weight of approximately 5000, compared to conventional heparin (UFH) with a molecular weight of approximately 15,000. Subsequently, the absorption, clearance, and activity of LMWH differ from UFH. Like the conventional forms of heparin, LMWHs produce their effect by binding to antithrombin (AT) and increasing antithrombin–mediated inhibition of synthesis and activity of coagulation factor Xa. However, LMWH, unlike conventional heparin, produces less inhibition of thrombin (factor IIa). LMWH's activity is described by the anti-factor Xa/anti-factor IIa ratio. For dalteparin, the ratio is 2.7 : 1 (conventional UFH ratio is 1 : 1). In people, LMWHs have several advantages compared to UFH and include greater anti-Xa/IIa activity, more complete and predictable absorption from injection, longer duration, less frequent administration, reduced risk of bleeding, and a more predictable anticoagulant response. However, in dogs and cats, the half-life of LMWH is much shorter than in humans, reducing some of this advantage. In dogs the half-life of dalteparin is approximately 2 hours; in cats it is estimated to be 1.5 hours, which requires much more frequent administration in either species to maintain anti-Xa activity compared to humans. Cats need higher doses and shorter dose intervals than people. Pharmacokinetic properties in horses were similar to humans (Schwarzwald et al., 2002).

Indications and clinical uses Dalteparin, like other LMWHs, is used to treat hypercoagulability disorders and prevent coagulation disorders such as thromboembolism, venous thrombosis, DIC, and pulmonary thromboembolism. Clinical indications are derived from uses of conventional heparin or extrapolated from human medicine. It is not possible to extrapolate doses from a unit-for-unit exchange with other heparins. There have been few clinical studies to examine efficacy of LMWH in animals. Previously published doses extrapolated from humans (100 units/kg q 12 h, SC) have been shown not to produce adequate and consistent anti-Xa activity in dogs and cats (Alwood et al., 2007).

When administering LMWH, aPTT and PT clotting times are not reliable indicators of therapy, although prolonged aPTT is a sign of overdosing. In people, anti-Xa activity is considered the preferred laboratory measure of LMWH activity. Peak anti-Xa activity occurs 2 hours after dosing, and the target range for anti-Xa activity should be 0.5–1.0 U/ml for cats and 0.4–0.8 U/ml for dogs. However, the anti-Xa activity assay is not available in most veterinary hospitals, is impractical, and has produced an inconsistent attainment of the targeted range in dogs (Lynch et al., 2014).

The formulations of dalteparin available are 2500 units anti-factor Xa (16 mg dalteparin sodium) per 0.2 ml in a single-dose syringe; 5000 units anti-factor Xa (32 mg dalteparin sodium) per 0.2 ml in a single-dose syringe; and 10,000 units anti-factor Xa (64 mg dalteparin sodium) per ml in a 9.5 ml multiple-dose vial. The multiple-dose vial should be used within 2 weeks of initial penetration.

Although the doses have not been tested for efficacy, the most commonly recommended dose for dogs is 150–175 U/kg q 8 h SC and, if monitoring is available, doses can be further adjusted. For cats, a dose of 100 units/kg SC every 12 hours failed to produce a sustained anti-factor Xa activity (Alwood et al., 2007) and higher doses of 150 units/kg q 4 h SC, to 180 units/kg q 6 h SC are recommended.

There is even less known for horses, but one dose that has been recommended is 50 units/kg/day SC. The dose may be increased in high-risk patients to 100 units/kg/day. In a study comparing dalteparin in horses to UFH, there were fewer adverse effects with dalteparin, and better efficacy for preventing thrombosis in the dalteparin treatment group (Feige et al., 2003). The dose administered (50 units/kg) produced the desired anti-Xa activity recommended for people (Schwarzwald et al., 2002; Feige et al., 2003).

Adverse reactions and precautions Although better tolerated than conventional heparin, bleeding can be a risk. LMWHs are associated with a lower incidence of heparin-induced thrombocytopenia in people, but heparin-induced thrombocytopenia from any form of heparin has not been a clinical problem in animals. If excessive anticoagulation and bleeding occur as a result of an overdose, protamine sulfate should be administered to reverse heparin therapy (discussed in Section Reversing Agent for Heparin: Protamine Sulfate).

If dalteparin is administered IM, it may produce a hematoma; therefore, SC administration is preferred. Because LMWHs are excreted by renal clearance in animals, if renal disease is present, the elimination will be prolonged. Rebound hypercoagulability may occur after discontinuation of heparin treatment; therefore, it may be advised to taper the dose slowly when discontinuing treatment.

Heparins interact with other drugs and it is not advised to mix these preparations with other injectable drugs. It should be used cautiously in animals that are already receiving other drugs that can interfere with coagulation, such as aspirin, clopidogrel, and warfarin. Although a specific interaction has not been identified, use cautiously in animals that may be receiving certain chondroprotective compounds such as glycosaminoglycans for treatment of arthritis.

Enoxaparin Sodium

In people, enoxaparin has largely replaced dalteparin for routine use. Enoxaparin (Lovenox) is characterized by a molecular weight of approximately 5000, compared to conventional heparin (unfractionated) with a molecular weight of approximately 15,000. Like dalteparin, the absorption, clearance, and activity of LMWH differ from UFH, and like dalteparin the mechanism of action is the same. The anti-factor Xa/anti-factor IIa ratio for enoxaparin is between 3.3 and 5.3 : 1 according to the USP standard, which is higher than dalteparin and higher than conventional UFH that has a ratio of 1 : 1. As with dalteparin, there are several advantages in people for enoxaparin over conventional UFH. But, as found with dalteparin, in dogs and cats the half-life of LMWH is much shorter than in humans, reducing some of this advantage. Similar to studies with dalteparin, cats require higher doses and shorter dose intervals than people (Alwood et al., 2007). In dogs the half-life of enoxaparin is approximately 5 hours (Lunsford et al., 2009); in cats it is estimated to be 1.9 hours, which requires much more frequent administration in either species to maintain anti-Xa activity compared to humans. In horses, the pharmacokinetics are similar to humans (Schwarzwald et al., 2002).

Clinical use Enoxaparin, like other LMWHs, is used for the same indications cited above for dalteparin. As for dalteparin, there have been few studies in animals to examine efficacy of LMWH, and doses extrapolated from humans are not appropriate for animals and are not expected to produce adequate and consistent anti-Xa activity in dogs and cats. As with dalteparin, do not interchange doses on a unit-for-unit basis with heparin or other low-molecular-weight heparins because they differ in manufacturing process, molecular weight distribution, anti-Xa and anti-IIa activities, units, and dosage.

When administering LMWH, aPTT and PT clotting times are not reliable indicators of therapy, although prolonged aPTT is a sign of overdosing. Anti-Xa activity is considered the preferred laboratory measure of LMWH activity in people, but this assay is not widely available for veterinarians, the test is impractical for veterinarians, and the use of this parameter is controversial in animals. Nevertheless, peak anti-Xa activity occurs 3–4 hours after dosing. If the test is available, the target range for anti-Xa activity should be 0.5–1.0 U/ml for cats and 0.5–2.0 U/ml for dogs.

Enoxaparin is available in 30 mg in 0.3 ml, 40 mg in 0.4 ml, 60 mg in 0.6 ml, 80 mg in 0.8 ml, 100 mg in 1 ml injection, 100 mg/ml injection, 120 mg per 0.8 ml, 150 mg/ml injection, and 300 mg per 3 ml. The most common dose for dogs is 0.8 mg/kg SC q 6 h and adjusted with monitoring if possible. At this dose, it consistently maintained target levels of ant-Xa activity (Lunsford et al., 2009). For cats the dose is 1 mg/kg SC q 12 h, up to 1.25 mg/kg SC q 6 h. A dose of 1.5 mg/kg every 6 hours SC was recommended by Alwood et al. (2007) because the lower dose failed to induce sustained anti-factor Xa activity. In horses, the dose used is 0.5 mg/kg q 24 h SC for prophylaxis and 1 mg/kg q 24 h SC for high-risk patients. For horses, 40 units/kg SC once daily has been recommended for prophylactic treatment and increased to 80 units/kg for horses at high risk (Schwarzwald et al., 2002).

Adverse reactions and precautions Although better tolerated than regular heparin, bleeding is a risk as it is with dalteparin. Other precautions and risks are the same as for dalteparin.

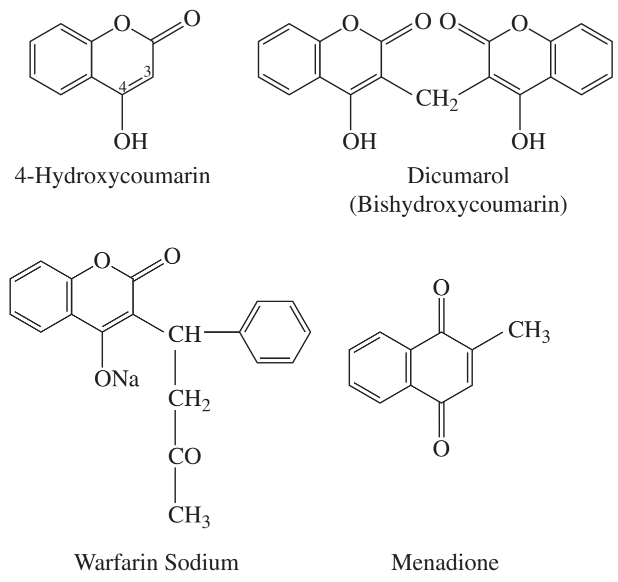
Reversing Agent for Heparin: Protamine Sulfate

Protamine sulfate is a low-molecular-weight protein found in the sperm of certain fish. It is strongly basic and combines with acidic heparin to form a stable inactive salt that prevents further anticoagulant activity of heparin. Protamine is used as an antagonist against heparin-induced hemorrhages. It also arrests action of heparin in vitro. Protamine also has some anticoagulant properties because of interference with the reaction of thrombin and fibrinogen. This would imply that the clinician must take care not to over-neutralize the action of heparin.

Protamine is available as a 1–2% solution. It is administered slowly by the IV route at a rate no greater than 50 mg over a 10-minute period. The dose is 1 mg protamine to neutralize 80–100 units of unfractionated heparin. This is equivalent to approximately 1–1.5 mg to antagonize each 1 mg heparin. The dose to antagonize dalteparin is 1.0 mg protamine for every 100 U dalteparin administered and for enoxaparin the dose is 1 mg protamine for every 1 mg enoxaparin. The dose may need to be adjusted to account for the lapse of time from administration; for example 30 minutes after heparin injection only 0.5 mg protamine may be required to antagonize each 1 mg heparin.

Warfarin and Coumarin Derivatives

Coumarin, normally present in some species of sweet clover, has little anticoagulant action. However, bishydroxycoumarin, a derivative of moldy or spoiled sweet clover, is responsible for hemorrhagic disease in cattle. This compound was synthesized by Link (1943–44). Other drugs have been synthesized with the


4-Hydroxycoumarin

Dicumarol (Bishydroxycoumarin)

Warfarin Sodium

Menadione

Figure 25.3 Structures of 4-hydroxycoumarin, dicumarol (bishydroxycoumarin), warfarin, and synthetic vitamin K (menadione).

4-hydroxycoumarin structure. Of the several coumarin derivatives, bishydroxycoumarin (dicoumarol) was the first oral anticoagulant. In 1948, a much more potent derivative was synthesized as 3-(α-acetonylbenzyl)-4-hydroxycoumarin, but is better known as warfarin. It was FDA approved in 1954 and has been in use ever since then. Warfarin sodium is the most common agent used in this group (marketed as Coumadin®), but all were developed from 4-hydroxycoumarin. The chemical structures of coumarin, dicumarol, warfarin, and menadione are shown in Figure 25.3. Bioavailability of warfarin is much greater than dicumarol because it is approximately 75,000 times more soluble in aqueous media. Warfarin, as well as other vitamin-K antagonists, are still used commonly as rodenticides because they produce fatal internal bleeding. Domestic animals can become inadvertently intoxicated through accidental exposure. Treatment of intoxication is with vitamin K, as discussed in Section Mechanism Of Action.

Mechanism Of Action

Warfarin and the other coumarin derivatives share one major pharmacological action: in vivo inhibition of blood coagulation mechanisms. This activity is achieved by inhibition of hepatic synthesis of vitamin K–dependent clotting factors, that is prothrombin and factors VII, IX, and X (Figure 25.1) (Hirsh, 1991). Therefore, unlike heparin, warfarin is inactive in vitro. The in vivo anticoagulant activity is apparent only after a latent period of at least several hours, which accounts for the time required for natural breakdown of circulating factors already present in the blood (Wajih et al., 2004). After warfarin administration is discontinued, anticoagulant effects can last for several days. This reflects the time necessary for reappearance of newly synthesized clotting factors. The half-life of existing clotting factors varies: factor VII, 6 hours; factor IX, 24 hours; factor X, 36–40 hours; and factor II, 50–60 hours. Because factor VII has the shortest half-life, the test that measures the activity of factor VII will have the greatest ability to detect either subtherapeutic or toxic dosages (PT clotting time and INR, discussed in Section In Vitro Anticoagulants and Clotting Tests). Because factor II has the longest half-life, full antagonist effects of warfarin may not be observed for several days.

The formation of functional clotting factors II, VII, IX, and X is dependent upon the presence of vitamin K. After the precursor proteins of these clotting factors have been synthesized in the liver, they must undergo carboxylation of terminal glutamic acid residues. The carboxylation results in the oxidative inactivation of vitamin K. The resulting vitamin K epoxide is then recycled by epoxide reductase so that it can again participate in the conversion of precursor proteins to functional clotting factors (Figure 25.4). Vitamin K antagonists exert their effect by inhibiting epoxide reductase. This results in a rapid depletion of vitamin K stores. The coagulation factors are still produced but are not functional. Vitamin K and warfarin are mutually antagonistic, and administration of vitamin K can reverse the anticoagulant effects produced by warfarin. The characteristics of warfarin and those of heparin are shown in Table 25.2.

Table 25.2 Pharmacological properties of heparin and warfarin anticoagulants

Characteristic	Heparin	Warfarin
Mechanism of action	Accelerates activity of antithrombin, which inhibits thrombin and activated factors VII, IX, X, XI, and XII	Inhibit hepatic synthesis of vitamin K-dependent clotting factors (II, VII, IX, and X)
Onset of action	Immediate	Delayed 12–24 hours
Duration of action	4 hours	2–5 days
Route of administration	Parenteral	Oral
Laboratory control test	Clotting time, partial thromboplastin time	Prothrombin time
Antidotal therapy	Protamine, fresh blood	Vitamin K, fresh blood or plasma
In vitro activity	Yes	No

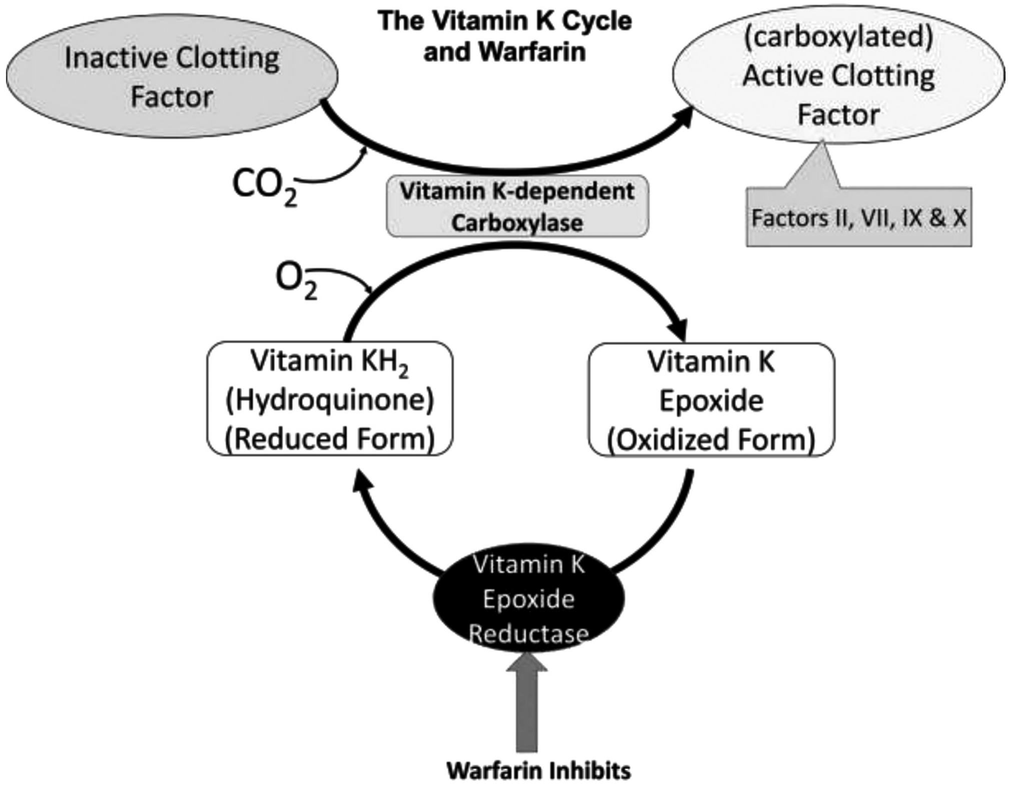

The Vitamin K Cycle and Warfarin
Inactive Clotting Factor
(carboxylated) Active Clotting Factor
CO_2
Vitamin K-dependent Carboxylase
Factors II, VII, IX & X
O_2
Vitamin KH_2 (Hydroquinone) (Reduced Form)
Vitamin K Epoxide (Oxidized Form)
Vitamin K Epoxide Reductase
Warfarin Inhibits

Figure 25.4 The vitamin-K cycle and the action of warfarin to inhibit the vitamin-K dependent clotting factors.

Pharmacokinetic Properties

The half-life of warfarin in people is long (36–42 hours), but shorter in animals. In dogs the half-life was 20–24 hours or 14.5 hours (depending on the study) (Neff-Davis et al., 1981). The half-life was 20–30 hours in cats (Smith et al., 2000a). In horses, the half-life was 13.3 hours (Thijssen et al., 1983). In all animals studied (including people) the protein binding is high – greater than 90% (often higher than 95%). Warfarin and other coumarin derivatives are consistently well absorbed orally. Reliable and consistent oral absorption is not associated with predictable activity. Subsequently, there is a large degree in the variability of action and a single fixed dose will not be appropriate for all patients. The variability is caused by differences among animals in metabolism, activity of cytochrome P450 (CYP450) metabolizing enzymes, genetic differences, a possible influence of diet composition (amount of vitamin K), and interaction with other drugs. As summarized in a review by Lesko (2016), in people there is greater than 10-fold intersubject variability in dose requirements. Much of this variability in people can be accounted for by variations in two genes. Genotyping for these genes is now available to test people so that doses of warfarin can be administered to people with greater precision. However, these tests are not available for animals.

Clinical Use, Administration, and Monitoring

Because of the variable action among animals, requirement for monitoring, and availability of other drugs, these agents are not used commonly in veterinary medicine today. There are there are no clinical trials that have shown efficacy for hypercoagulation states in animals. In older guidelines, warfarin was recommended for small animals to prevent thromboembolism from a variety of diseases, especially heart disease. In horses, warfarin was once recommended to treat navicular disease, although it is no longer popular for this use.

The high variability in individual and species response to warfarin necessitates laboratory monitoring of prothrombin activity for safe and effective clinical use. Contraindications include bleeding from any cause, purpura of any type, or a severe state of malnutrition.

Warfarin response can be highly variable among animals. Pharmacokinetic studies have attempted to correlate plasma pharmacokinetics with clinical response (prothrombin time [PT]) (Neff-Davis et al., 1981). However, such a correlation has been difficult to demonstrate. A specific dose and plasma concentration that produces an effective prolongation of prothrombin time in one patient may not be effective in another individual. Because of the variation in response, adjustment of doses by monitoring bleeding times in treated animals is needed, but rarely performed clinically.

Because the optimum dose should be identified for each patient, the best method to monitor warfarin therapy is with the one-stage PT and INR (discussed in Section In Vitro Anticoagulants and Clotting Tests). In animals, the dose is adjusted to maintain PT at 1.5 to 2 times normal (or INR of 2–3). Warfarin sodium is available in 1, 2, 2.5, 4, 5, 7.5, and 10-mg tablets. Warfarin sodium is soluble in water. It is light sensitive and should be packaged in containers that protect the medication from light and moisture. Solutions should have a pH greater than 8 to maintain solubility. Some tablets do not have the drug distributed evenly; therefore, uneven doses can result from splitting tablets. When dividing tablets for treatment, it is best to crush up a whole tablet into a powder and divide the doses equally from the powder.

For a rapid effect in dogs consider a loading dose of 6 mg per dog, once daily, for two treatments in order to achieve the targeted INR (Monnet and Morgan, 2000). A typical dose for dogs is 0.1–0.2 mg/kg q 24 h PO. Start with this dose q 12 h for the first 2–4 days because of a lag time before maximum effect is observed. For cats, start with 0.25 to 0.5 mg/cat/day and adjust dose based on bleeding time assessment (equivalent to 0.06 to 0.09 mg/kg). Adjust dose by monitoring clotting time. When cats were administered 0.5 mg/kg the maximum response occurred in 24–48 hours (Smith et al., 2000b). The median dose to produce therapeutic concentrations was 0.06–0.09 mg/kg/day. However, the concentration needed to produce the targeted INR value of 2–3 was highly variable among cats. There was wide variation in pharmacokinetics and response.

For horses, administer 0.02 mg/kg q 24 h PO (9 mg per 450 kg of body weight [1000 pounds]). Increase this dose gradually (by increments of 20%) until a 2 to 4-second increase in PT bleeding time is achieved. Allow 7 days between changes in dose. After a dose of 0.75 mg/kg the effect of warfarin in horses was noticed only after 60 hours and lasted for approximately 30 hours (Thijssen et al., 1983).

Adverse Effects and Precautions

Adverse effects are attributable to decreased blood clotting. In people, it is among the top 10 drugs causing serious or fatal adverse drug reactions. (Dabigatran, discussed in Section Thrombin Inhibitors**,** is also in the top 10.) Spontaneous bleeding can result in blood loss, hemoperitoneum, hemarthrosis, gastrointestinal bleeding, epistaxis, and excessive bleeding from trauma or surgery.

Multiple drugs and some foods may affect warfarin's action (Daly and King, 2003). Some of these that may potentiate warfarin's action include aspirin, clopidogrel, chloramphenicol, phenylbutazone, ketoconazole, and cimetidine. Drugs that induce liver enzymes may accelerate metabolism (e.g., phenobarbital, griseofulvin, rifampin). Drug interactions are possible if administered with other highly protein-bound drugs, but such reactions are poorly documented in animals. Drug interactions are also possible with trimethoprim, sulfonamides, and metronidazole. Do not administer with some cephalosporin drugs (particularly those with *N*-methylthiotetrazole, NMTT) because cephalosporins may induce bleeding through antivitamin K–dependent mechanisms.

Long-acting Rodenticides

In addition to warfarin, there are other rodenticide derivatives, sometimes called second-generation rodenticides. These include *bromadiolone, brodifacoum, diphenadione, chlorophacinone*, and *pindone*. These are not used therapeutically but are found in the long-acting rodenticides. Animals may become inadvertently exposed through poisoning. Because their effects can persist for many days, and even weeks, treatment with warfarin antagonists (vitamin K) must continue for as long as 6 weeks until clotting times return to normal ranges. Specific instruction on treatment of poisoning from these rodenticides is found in internal medicine and toxicology textbooks, or by contacting an animal poison control center.

Thrombin Inhibitors

The inhibition of thrombin (Figure 25.1) is a key role in producing anticoagulant effects Thrombin is a logical target because it is involved in the final step in coagulation and thrombin is also an activator of platelets. Thrombin also stimulates further coagulation by activating cofactors V and VIII (Table 25.1). One of the first direct thrombin inhibitors was ximelagatran. It was briefly on the market until the hepatic toxicity associated with its use required withdrawal.

Dabigatran etexilate (Pradaxa®) is the most promising of the thrombin inhibitors at this time (Table 25.3). There is little information on its use in animals. In people it has oral bioavailability of only 6%. The ester dabigatran etexilate is converted to dabigatran quickly by esterases. More detailed pharmacokinetic features are listed in the paper by Gong and Kim (2013). Most of dabigatran is eliminated unchanged in the urine; therefore, it is less subject to drug metabolizing interactions.

There has not been published reports of clinical use of dabigatran in veterinary medicine. Some use, and doses, have been extrapolated from the experience in human medicine (see Table 25.3 for dosages.)

Table 25.3 New anticoagulant drugs

	Drug		
	Dabigatran etexilate	**Rivaroxaban**	**Apixaban**
Brand name	Pradaxa	Xarelto	Eliquis
Target	Thrombin	Factor Xa	Factor Xa
Prodrug	Yes	No	No
Monitoring required	No	No	No
Half-life (people)[a]	12–17 hours	5–9 hours	8–15 hours
Human dose	150 mg twice daily	20 mg once daily	5 mg twice daily
Interactions possible?[a,b]	p-glycoprotein inhibitors and inducers[c] may affect oral absorption	CYP 450 inhibitors, and p-glycoprotein inhibitors[c]	Potent CYP 450 inhibitors[c]

[*] Other pharmacokinetic features and drug interactions in people can be found in the review by Gong and Kim (2013).

[†] Interactions possible are listed for people. These are possible in animals but specific studies have not been performed.

[‡] Examples of inhibitors may include ketoconazole, diltiazem, and macrolide antibiotics.

Examples of inducers include rifampin and phenobarbital.

One of the disadvantages of new anticoagulant drugs has been bleeding problems and the inability to reverse these effects. On the other hand, warfarin is effectively reversed with vitamin K administration. However, recently a new reversal agent became available for dabigatran, idarucizumab (Praxbind®). This agent is approved for urgent reversal of the anticoagulant effect of dabigatran etexilate. Idarucizumab is the first such specific reversal agent to become available for one of the new oral anticoagulants.

In people, more adverse reaction reports were generated with the use of dabigatran than the other new agents (listed in Section Factor Xa Inhibitors). There is a relatively high rate of gastrointestinal problems from oral dabigatran. It requires an acid environment for oral absorption and an acidifier is included in the formulation. This has caused dyspepsia in some people and the need to switch to another medication.

Factor Xa Inhibitors

Rivaroxaban and Apixaban

The inhibition of the active form of factor X (Xa) is also an attractive target for treating clotting disorders. Factor Xa plays a key role in the activation of the clotting cascade (Figure 25.1). The structure of Factor Xa is similar, but not identical, in dogs and humans, indicating that Factor Xa inhibitors used in people might be effective in dogs. Direct factor Xa inhibitors include rivaroxaban (Xarelto®) and apixaban (Eliquis®), both of which have become popular in human medicine (Table 25.3). The newest agent in this group is edoxaban (Savaysa®). In theory, the inhibition of Xa produces less bleeding adverse events than inhibition of thrombin, but this has not been shown in human trials.

These new agents have several advantages over warfarin. The pharmacokinetics are predictable and a fixed dose may be administered to produce results without routine monitoring as required for warfarin. They are not affected by dietary changes, and are less subject to drug interactions, although some are possible. More detailed pharmacokinetic features are listed in the paper by Gong and Kim (2013).

Dabigatran (discussed in Section Thrombin Inhibitors) and the factor Xa inhibitors are primarily for oral use to prevent thromboembolism associated with atrial fibrillation in people. The clinical effectiveness of these three new agents appears similar (Potpara and Lip, 2011; Mantha et al., 2013). These agents are also used for venous thrombosis, such as deep vein thrombosis, but only rivaroxaban is approved for this indication.

There have been some limited reports of the use of rivaroxaban and apixaban in dogs and cats. Dogs with immune-mediated hemolytic anemia (IMHA) are prone to thromboembolism and they typically have poor response to antiplatelet therapy, heparin, and LMWH (Kidd and Mackman, 2012). Because of lack of other alternatives for treating IMHA-associated thromboembolism, new agents such as rivaroxaban have been examined. Rivaroxaban was administered to dogs at an average dose of 0.89 mg/kg once daily (Morassi et al., 2016). It was well tolerated in this small group of dogs. There were no significant differences among treatment groups; therefore, it was not possible to determine if rivaroxaban was more beneficial than standard antiplatelet therapy. In limited case reports (Yang et al., 2016) rivaroxaban was administered to dogs for treatment of thrombotic complications at oral doses of 0.5–1 mg/kg once daily, with mixed results.

The pharmacokinetics and pharmacodynamics of oral apixaban have been studied in healthy cats (Myers et al., 2015). The half-life after oral administration of 0.2 mg/kg was approximately 3 hours and bioavailability was 86%. The half-life in other species is similar, but was longer in people (Table 25.3). It was an effective inhibitor of factor Xa in the cats and activity was correlated with plasma drug concentrations. However, because of the short half-life, it would be difficult to maintain anticoagulant activity with twice-daily administration. Oral absorption is at least 50% in dogs (He et al., 2011).

The most detailed study with rivaroxaban has been in cats (Dixon-Jimenez et al., 2016). Cats were treated at three doses (1.25, 2.5, and 5 mg/kg) for pharmacokinetic studies. Oral doses were also administered every 12 or 24 hours for 3, 7, or 28 days for coagulation analysis. The doses were well tolerated and there were dose-related changes in clotting times and factor Xa inhibition. The half-life of rivaroxaban in cats was 7.55 hours (± 3.1 hours). At the doses studied in cats, the anti-Xa activity was not maintained in the human therapeutic range throughout the duration of a 12- or 24-hour interval. Until other studies are available, doses higher than 2.5 mg/kg twice daily are recommended for testing.

Rivaroxaban also has pharmacokinetics that were unfavorable in dogs (Weinz et al., 2005). Although oral absorption is good, the half-life is short (1 hour). Despite the short plasma half-life, a study in healthy Beagle dogs that measured anticoagulant activity concluded that a dose of 2 mg/kg twice, at 8 hour intervals maintained anticoagulant activity for a 24 hour interval (Conversy et al., 2017).

Adverse Effects and Precautions

One of the disadvantages of new anticoagulant drugs has been bleeding problems and the inability to reverse the effects. Warfarin can be reversed with vitamin K. A new reversal agent for dabigatran (idarucizumab) has only recently become available, but has not been used in animals. A new agent, andexanet, is being developed for antagonizing effects of Factor Xa inhibitors. Other adverse effects are derived from the human use, because there has not been enough experience with these drugs to know the adverse effects in animals. In short-term studies they have been well tolerated. Because of the short half-life of these drugs compared to warfarin, there is a concern that missed doses could lead to thromboembolic risks.

Agents to Reverse Warfarin/ Coumarin

Vitamin K

Vitamin K is a fat-soluble vitamin that is found in a variety of plants and is also produced by microorganisms. Its main therapeutic usage is in the treatment of vitamin K antagonist rodenticide intoxication. Vitamin K is a cofactor used to synthesize coagulation factors in the liver (factors II, VII, IX, and X) (Figure 25.4). It also is vital for the production of active protein C and protein S, both of which have anticoagulant effects.

Vitamin K_1 (phytonadione) is present in plants, vitamin K_2 (menaquinone) is produced by microorganisms, and vitamin K_3 (menadione) is a synthetic derivative (Figure 25.3). Vitamin K_1 is the most immediately available fat-soluble vitamin used to treat coagulopathies caused by anticoagulant toxicosis (warfarin or other rodenticides). These anticoagulants deplete vitamin K in the body, which is essential for synthesis of clotting factors.

Vitamin K_1 is also known as phytonadione and phylloquinone (phytomenadione is the British spelling of phytonadione). Vitamin K_2 is also known as menaquinone. Vitamin K_3 is known as menadione. Vitamin K_3 is a synthetic analogue and is not equivalent to Vitamin K_1, and is not recommended for clinical use. Vitamin K_3 has demonstrated poor efficacy in veterinary patients as well as demonstrating toxic side effects (Alstad et al., 1985; Fernandez et al., 1984).

Phytonadione and phytomenadione are synthetic lipid-soluble forms of vitamin K_1. Menadiol is vitamin K_4, which is a water-soluble derivative converted in the body to vitamin K_3 (menadione). Other forms of vitamin K may not be as rapidly acting as vitamin K_1; therefore consider using a specific preparation.

Vitamin K_1 is actively absorbed in the small intestine. Vitamins K_2 and K_3 are passively absorbed in the ileum and colon. Solubilization by bile acids is required for absorption of vitamins K_1 and K_2. Biotransformation in the organism is required for vitamin K_3 to become active (Mount, 1982).

Indications and clinical uses The phytonadione form of vitamin K_1 is used to treat coagulopathies caused by anticoagulant toxicosis (warfarin or other rodenticides). In large animals, it is used to treat sweet clover poisoning. Vitamin K_1 is preferred for acute therapy because it is more highly bioavailable. When treating poisoning caused by rodenticides, consult a poison control center for the protocol if the specific rodenticide is identified. Some long-acting rodenticides require prolonged treatment (6 weeks) and identification of the specific agent is important. Because this is a fat-soluble vitamin, the oral forms of vitamin K_1 should be administered with food to enhance absorption. The preferred route for initial treatment is SC, but IM also can be used. When treating for poisoning by second-generation rodenticides, which have long half-lives, 6 weeks of therapy may be necessary. If an injection is used, it can be diluted in 5% dextrose or 0.9% saline but not other solutions. Although vitamin K_1 veterinary labels have listed the intravenous route for administration, these labels have not been approved by the FDA. Therefore, avoid intravenous administration of vitamin K_1.

Monitoring treatment of poisoning is important to ensure proper duration. Monitoring the clotting times (as discussed in Section Clinical Use, Administration, and Monitoring) can be used as a guide. Factor VII is the vitamin K-dependent clotting factor with the shortest half-life and is also the factor predominantly measured by PT. As a result, PT values will change before other clotting time tests.

The typical dose of Vitamin K_1 for dogs and cats for treatment of short-acting rodenticides is 1 mg/kg/day for 10–14 days IV, SC, or oral. For treatment of long-acting rodenticides administer 2.5–5 mg/kg/day for 3–4 weeks IM, SC, or PO. For cattle, calves, horses, sheep, and goats, the dose is 0.5–2.5 mg/kg SC or IM.

Adverse reactions and precautionary information In people, a rare hypersensitivity-like reaction has been observed after rapid intravenous injection. This reaction may be caused by histamine release from a reaction to the drug vehicle, Polysorbate 80. Signs resemble anaphylactic shock. These signs also have been observed in animals. To avoid anaphylactic reactions, do not administer IV. Reactions from intramuscular injection, such as hematoma, may occur in animals with coagulopathies.

Agents for Treating Coagulopathies

Desmopressin Acetate

Desmopressin acetate (DDAVP) is a synthetic analog of vasopressin, and is used in the treatment of central diabetes insipidus and to transiently elevate levels of vWF. (See Chapter 26 for endocrine uses.) Its actions to increase vWF are via the V_2 receptor rather than the V_1 receptor. The V_1 receptor is the vascular receptor that is responsible for vasoconstriction. The elevation in vWF allows surgical procedures to be performed and aids in the control of capillary bleeding from wounds in humans and animals with certain forms of von Willebrand disease. In comparison to vasopressin, DDAVP has minimal pressor effects. When DDAVP is administered, it releases stored vWF from endothelial cells and macrophages and produces a rapid rise in vWF levels, with larger, more-active multimers predominating (Kraus et al., 1989; Johnstone and Crane, 1986). This effect is reduced if the drug is given repeatedly, because the storage pools will be depleted. The duration of elevation is approximately 2 hours (Mansell and Parry, 1991). Though the elevation in vWF levels found in dogs is considerably lower than in humans, clinically a decrease in buccal mucosal bleeding time was seen (Kraus et al., 1989), but in some dogs only a minimal response was noted. The recommended dosages for dogs for von Willebrand's disease treatment is 1 μg/kg (0.01 ml/kg), administered SC, or diluted in 20 ml of saline and administered over 10 minutes IV.

Antiplatelet Drugs

Platelets have a central role in the initiation and propagation of thrombus formation (Figure 25.2). They release a variety of substances that promote coagulation, and they also form the initial platelet plug. Platelets adhere and aggregate in response to a myriad of stimuli. A central mechanism in aggregation is alteration of cAMP concentrations in the platelet itself. Increased cAMP is inhibitory to aggregation, while decreased cAMP is proaggregatory. Our ability to study antiplatelet drugs is enhanced by the improved availability of platelet activity tests such as thromboelastography (TEG), platelet aggregometry, and the Platelet Function Analyzer 100. These test systems are available in research laboratories of veterinary scientists who study these drugs and in many veterinary hospitals that treat critically ill patients with clotting disorders. The specific tests and their interpretation will not be discussed here but are available in various internal medicine and critical care books.

A variety of drugs can be used to reduce platelet function. Clinically this should be beneficial in the prevention of thrombotic disease, especially if the clot forms on the arterial side of the vascular system. The efficacy of these drugs in the prevention of myocardial infarction and stroke in humans has been well established and some of the drugs and strategies have been adapted from human medicine. The most common indications in veterinary medicine are to prevent thrombi associated with feline cardiomyopathy and to reduce the severity of pulmonary endarteritis associated with heartworm disease. Although work in the 1980s promoted the use of aspirin as an antiplatelet drug for alleviating the proliferative changes in arteries associated with heartworm infestation (Boudreaux et al., 1991; Keith et al., 1983; Schaub et al., 1983), this indication no longer has support. The American Heartworm Society no longer recommends aspirin treatment for canine heartworm disease patients because convincing evidence of clinical benefit is lacking and some research indicating that aspirin treatment may produce harm (https://www.heartwormsociety.org/). Platelet inhibition is potentially beneficial for other diseases such as membranous glomerulonephritis, mild DIC, pulmonary thromboembolism, and vasculitis, but protocols are not fully developed for all these indications. In horses, platelet inhibition may be of benefit in the treatment of thrombotic disorders such as laminitis and navicular disease.

Aspirin

A commonly used antiplatelet drug is aspirin (acetylsalicylic acid, ASA). Aspirin is a nonsteroidal antiinflammatory drug (NSAID) that inhibits the activity of cyclooxygenase (discussed in detail in Chapter 20.). Other NSAIDs, discussed in Chapter 20, inhibit the enzyme cyclooxygenase, but are not as specific as aspirin for COX-1, the predominant enzyme found in platelets. Aspirin is unique in that even at low doses it causes irreversible inhibition of platelet cyclooxygenase. The acetyl-group on aspirin acetylates cyclooxygenase in platelets and blocks the synthesis of thromboxane A_2 (TXA_2). By contrast, prostacyclin (PGI_2) synthesis by the vascular endothelium is mediated by COX-2 and is not affected by low doses of aspirin. Thromboxane A_2 and PGI_2 have opposing actions on platelet function and vascular smooth muscle tone. Prostacyclin is a potent vasodilator and inhibitor of aggregation, while TXA_2 is a vasoconstrictor and stimulates platelet aggregation.

After oral administration, aspirin is absorbed into the portal vein where most of the platelet acetylation of COX-1 in platelets takes place. Subsequently, aspirin enters the liver where it is rapidly converted to salicylic acid. Therefore, most acetylation in platelets occurs in the portal circulation where aspirin levels are the highest and acetylation of COX-1 can occur. The COX-2 enzyme in the vascular endothelium that synthesizes PGI_2 is exposed to primarily salicylic acid, not aspirin. Because platelets do not synthesize new enzymes (platelets have no nucleus), this results in decreased platelet aggregation throughout the lifespan of the platelet. Aggregation response will return to normal as new platelets enter the bloodstream from the bone marrow. If endothelial cell cyclooxygenase activity (PGI_2) is inhibited, it recovers more rapidly because new enzyme can be synthesized. Theoretically, at low aspirin doses this selective inhibition of COX-1 over COX-2 is maintained, producing a specific antiplatelet effect, but at high doses the selectivity is diminished. In addition, low dosages should minimize the gastrointestinal side effects that can occur following aspirin administration. This theoretical basis for antiplatelet aspirin dosages notwithstanding, the goal of finding an optimum low aspirin dose has proven elusive. Studies of the effect of aspirin on ADP or collagen-induced canine platelet aggregation do not reliably show a decrease in aggregation and, consequently, use of aspirin alone may be suboptimal for thromboprophylaxis in some dogs (Brainard et al., 2007). Experimental infestation and embolization with heartworms lead to a pronounced increase in the amount of aspirin needed to maintain this level of inhibition (Boudreaux et al., 1991).

Dosage recommendations for platelet inhibition vary widely. In healthy dogs a dose of 0.5 mg/kg twice daily aspirin was found to be more effective than higher doses (Rackear et al., 1988). But many dogs do not respond to low-dose aspirin and as many as 30% are not responsive. More frequent dosing was needed to produce a consistent response (Rackear et al., 1988).

In cats 25 mg/kg twice weekly has been found to inhibit platelet aggregation without evidence of toxicity (Greene, 1985). However, aspirin produces inconsistent antiplatelet effects in cats. Platelets collected from cats ex vivo after treatment with aspirin (5 mg/kg) had decreased thromboxane production (TXA_2) but platelet aggregation was not affected. Doses of 5 mg or 20.25 mg to cats every 72 hours produced inconsistent response and was not as effective as clopidogrel (Ho et al., 2016). Treatment with 81 mg per cat every 3 days has not been as effective for preventing arterial thromboembolism compared to clopidogrel (Hogan et al., 2015).

Experimental studies in horses showed that oral aspirin (12 mg/kg) significantly increased bleeding times for 48 hours posttreatment (Trujillo et al., 1981). A lower dose (4 mg/kg) prolonged bleeding times for 4 hours posttreatment (Cambridge et al., 1991).

Clinical use and dosages Despite the inconsistent results and variable activity, aspirin continues to be used by veterinarians as an antiplatelet agent. Although the optimum dose is not determined, low doses of aspirin are routinely used for antiplatelet therapy. Addition of other antiplatelet drugs such as clopidogrel (Plavix®, discussed in Section Clopidogrel) provides more effective inhibition and sometimes aspirin and clopidogrel are used together. Clinical doses are varied because of inconsistency of experimental results. Doses have ranged from 0.5 mg/kg every 12 hours, up to 5–10 mg/kg. Doses for cats have ranged from 5 mg per cat q 72 h, to 81 mg per cat q 72 h. In cats, a dose of 81 mg per cat is common (size of a typical children's aspirin tablet) every other day for convenience. A study to monitor long-term management of cats at risk for arterial thromboembolism did not identify a difference between a high dose (81 mg per cat) and a low dose (5 mg per cat) every 72 hours (Smith et al., 2003).

Clopidogrel

Clopidogrel is structurally related to ticlopidine, which exerts similar antiaggregation mechanisms. Clopidogrel (Plavix) is a prodrug that undergoes biotransformation into its active metabolite (Brainard et al., 2010). The active metabolite is a P2Y12 receptor inhibitor, thereby preventing ADP from exerting its strong cyclic AMP-dependent aggregating action (Figure 25.2). Its onset of action is therefore slow, but it seems to have fewer objectionable side effects than does ticlopidine. Clopidogrel is commonly used in conjunction with aspirin, and it has been approved for use in humans to prevent recurrence of stroke and myocardial infarction secondary to thrombus formation.

Clopidogrel is primarily metabolized by two main pathways, one leading to the formation of the inactive carboxylic acid metabolite (SR26334), and one leading to the active metabolite, a thiol derivate, through formation of 2-oxo-clopidogrel (Pereillo et al., 2002). This active metabolite is responsible for the antiplatelet effects (Savi et al., 2000). In humans, conversion to the active metabolite occurs via cytochrome P450 enzymes, including CYP2C19, CYP3A4, CYP1A2, and CYP2B6 (Mega et al., 2009). CYP2C19, considered the main enzyme in people, can be inhibited by some drug therapies, such as proton pump inhibitors (omeprazole) (Mega et al., 2009; Simon et al., 2009). Variable results for clopidogrel efficacy in people have been attributed to CYP2C19 polymorphisms (Samant et al., 2017). Although dogs are known to have CYP2C19, CYP3A4, CYP1A2, and CYP2B6 activity (Chauret et al., 1997), it is not known if dogs share the same metabolic pathway for clopidogrel as humans. Administration of omeprazole to dogs did not affect clopidogrel antiplatelet activity (discussed in Chapter 46). In humans, the active metabolite is subsequently converted to an inactive metabolite. Concentrations of the parent drug and active metabolite are usually undetectable in plasma (Brainard et al., 2010). Therefore, measurement of the inactive, carboxylic acid metabolite has been used as a surrogate marker to assess clopidogrel pharmacokinetics (Bahrami et al., 2008). In people, the inactive carboxylic acid metabolite represents 85% of the circulating metabolite.

Clinical use Because of the inconsistency of aspirin as an antiplatelet drug in animals, and the lower cost of the human form of clopidogrel after it became available as a generic tablet, interest in veterinary use has increased. It is used as an antiplatelet drug for patients with heart disease, thromboembolic disorders, heartworm disease, immune-mediated hemolytic anemia, and cats at risk of cardiogenic arterial thromboembolism. This drug has been safely administered to cats, rabbits, dogs, and calves (Brainard et al., 2007, 2010; Hogan et al., 2004; Wong et al., 2009; Coomber et al., 2006; Hong et al., 2005).

The use of clopidogrel is now common in dogs and cats. In dog, cats, and horses, oral administration has produced significant inhibitory effects on platelets that are superior to aspirin. A dose of either 0.5 or 1 mg/kg oral per day in dogs consistently produced a rapid and effective inhibition of canine platelet aggregation responses to both ADP and collagen. The effect of clopidogrel on the platelets of dogs appears to be irreversible (Brainard et al., 2010). In dogs, a dose of either 0.5 or 1.0 mg/kg, decreased ADP-induced platelet aggregation occurs for 3 days after discontinuation of drug administration in some dogs and longer than 7 days in others. Effects also persisted in cats for 3 days after administration. At 2 mg/kg orally, q 24 h, clopidogrel significantly suppressed platelet activity in horses, which persisted for 6 days after the last dose.

The sustained activity of clopidogrel may allow intermittent dosing (for example every other day) in clinical patients. The effect on ADP-induced aggregation was most profound, indicating that the drug is able to exert an effect at the canine $P2Y_{12}$ ADP receptor. In some dogs (which may have variants of the $P2Y_{12}$ receptor), clopidogrel activity may be absent or diminished, similar to responses reported in humans with polymorphisms in this receptor (Feher et al., 2010).

The tablet is a fixed dose of 75 mg. Therefore, either a whole tablet, or fraction of a tablet is used for treatment to simplify dosing. For example, the dose in cats is 18.75 mg per cat (1/4 tablet) q 24 h PO. Smaller doses may be effective but have not been evaluated in cats. For dogs a dose of 1–2 mg/kg q 24 h PO is used. An oral loading dose may be given at 2–4 mg/kg followed by 1–2 mg/kg q 24 h PO. (In some cases, a higher oral loading dose of 10 mg/kg has been used.) For horses, the loading dose is 4 mg/kg, followed by 2 mg/kg PO q 24 h.

Ticlopidine

Ticlopidine (Ticlid®) is also a $P2Y_{12}$ receptor inhibitor. Because of the availability of clopidogrel and more extensive information regarding the effects and dosage regimens in animals, ticlopidine is rarely used in veterinary medicine. Ticlopidine should not be used in cats because it produces adverse reactions.

Fibrinolytic Agents

Thrombolytic therapy is not preformed commonly in veterinary medicine. Use of these agents is expensive, and there is little evidence available to show that the benefits outweigh the risks and expense. Although it is an important component of the emergency treatment in people for acute ischemic strokes, myocardial infarction, deep vein thrombosis, and pulmonary thromboembolism, it has not been very successful in animals. The most common clinical presentations where this treatment is considered is feline arterial thromboembolism (Fuentes, 2012; Lunsford and Mackin, 2007) and canine pulmonary thromboembolism. These conditions are typically secondary to other problems (heart disease, heartworm disease, autoimmune disease) (Thompson et al., 2001). Pharmacological acceleration of fibrinolysis involves drugs that enhance the conversion of the inactive precursor plasminogen to the active fibrinolytic enzyme plasmin (Armstrong et al., 2003). Plasminogen exists in two phases, the plasma or soluble phase found in the circulating blood and the gel phase bound to fibrin in the formed clot. Thus when a plasminogen-activating agent comes in contact with the clot, fibrin-bound gel-phase plasminogen is activated to plasmin locally with selective fibrinolysis. There is increased tendency for systemic bleeding if, instead, soluble-phase plasminogen circulating in systemic blood is also activated. Plasmin formation would then occur throughout the circulation rather than being localized to the formed clot. Indeed, the presence of plasmin in peripheral blood indicates a pathological fibrinolytic state. Such a condition reflects over-activation of plasminogen, which overcomes the neutralizing capacity of an endogenous antagonist to plasmin called α_2-antiplasmin.

Streptokinase

Streptokinase is a stable, vacuum-dried powder containing enzymes produced by β-hemolytic *Streptococcus*. Streptokinase complexes with plasminogen, yielding plasmin. Streptokinase converts both circulating plasminogen as well as plasminogen associated with a thrombus. Therefore, systemic administration can produce wide-spread inhibition of clotting.

The most common use recorded in veterinary medicine is treatment of arterial thromboemboli in cats and pulmonary thromboembolism in dogs (Moore et al., 2000; Fuentes, 2012; Killingsworth et al., 1986; Thompson et al., 2001). A dose of 90,000 units administered IV to cats, followed by 45,000 units per hour, produced a systemic fibrinolytic state but did not reduce size of clots (Killingsworth et al., 1986). An average dose of 18,857 units/kg administered to cats with arterial thromboembolism may have provided a benefit in some cats but there was not a comparison group to compare effects of nonstreptokinase treatment (Moore et al., 2000). Some cats developed life-threatening hyperkalemia after treatment. There was no relationship between survival and dose administered. In other case reports reviewed by Lunsford and Mackin (2007), describing the use of streptokinase in cats, there were serious complications associated with the use, including death. The explanation for treatment failure is that cats with arterial thromboembolism have clinical signs caused by vasoactive substances (e.g., thromboxane and serotonin), and not directly attributed to the clot. Dissolving the clot does not mitigate the effects of the vasoactive substances, and can lead to reperfusion injury.

Streptokinase has had limited use in dogs, primarily for pulmonary thromboembolism. Published studies have been based on small numbers and not enough to determine if it is successful (Thompson et al., 2001). Doses administered have been 90,000 units per dog, followed by 45,000 units per dog, IV. Based on these experiences, until more information becomes available streptokinase treatment cannot be recommended as a routine treatment. It has also been used in large animals, but clinical study results are lacking. The daily dose for large animals is 5,000–10,000 units/45 kg.

Urokinase is isolated from fetal kidney cells in tissue culture. It has a similar effect as streptokinase, but there has been no recorded successful use in veterinary medicine.

Tissue Plasminogen Activator

Tissue-type plasminogen activator (t-PA) predominantly exerts its effect in association with fibrin clots. It binds to fibrin in a thrombus and converts the entrapped plasminogen to plasmin, thereby initiating local fibrinolysis. Unlike other plasminogen activators, t-PA does not readily induce a systemic fibrinolytic state and has less systemic bleeding problems than streptokinase. Therefore, the safety advantage of t-PA over streptokinase makes this a more attractive treatment. The currently available commercial product is recombinant DNA produced human-type tissue plasminogen activator (often referred to as rt-PA). This product, called Aletplase®, is produced by recombinant DNA technology synthesized using the complementary DNA (cDNA) for natural human tissue-type plasminogen activator (t-PA) obtained from an established human cell line.

There are only a few papers that have reported on the experience of administering t-PA to animals, mostly in cats with arterial thromboembolism (Thompson et al., 2001; Lunsford and Mackin, 2007). In cats with thromboembolism, a dose of 0.25–1 mg/kg per hour for a total dose of 1–10 mg/kg was associated with resolution of clots in some cats. Although intravenous administration of t-PA resulted in a rapid return of function in some cats, 50% of the animals treated died acutely after treatment (Pion, 1988). This was attributed to the rapid reperfusion of the rear extremities, which resulted in reperfusion-induced hyperkalemia and heart failure. There is no other clear evidence that supports the use of t-PA for treatment in cats (Lunsford and Mackin, 2007). The use in dogs has been reported only in isolated cases (1 mg/kg IV) and produced clinical benefits in these instances (Thompson et al., 2001).

Antifibrinolytic Drugs

Fibrinolysis produces breakdown of fibrin clots through conversion of plasminogen to plasmin. Ordinarily, in normal conditions there is a balance between clot-forming processes and fibrinolysis. Excessive fibrinolysis can lead to excessive bleeding disorders.

Drugs available for treatment of hyperfibrinolytic conditions are aminocaproic acid and tranexamic acid. These are synthetic lysine compounds that decrease fibrinolysis by binding to fibrin and competing for lysine-binding sites to inhibit plasminogen and plasmin. Aminocaproic acid prevents the association of fibrin with plasminogen. Concentrations needed to completely inhibit fibrinolysis in canine plasma after induction of hyperfibrinolysis were determined by Fletcher et al., (2014). Aminocaproic Acid (Amicar®), is available as 100- and 500- mg tablets, 250 mg/ml syrup, and 250 mg/ml for IV injection. There is limited information on the use of these products in animals. They are used anecdotally, but uses and doses are extrapolated from human medicine. In research animals, 100 mg/kg or 20 mg/kg was administered orally to dogs and blood samples collected for measurements of clot strength and clot lysis (Brown et al., 2016). In that study, administration of aminocaproic acid inhibited fibrinolysis at all doses tested. The 100 mg/kg dose was more effective in dogs than 20 mg/kg, and it was concluded that 100 mg/kg oral every 6 hours is recommended for patients with hyperfibrinolysis. On the other hand, a case study on the use of aminocaproic acid in 122 dogs with potential bleeding disorders used the lower dose of 18 mg/kg (average dose) and examined the need for red blood cell transfusions. This clinical study could not reach any conclusions regarding efficacy. The drug dose was not associated with improvements in packed cell volume. As expected, the most common adverse effect in people is thrombosis formation during treatment.

The other antifibrinolytic agent used is tranexamic acid. It is an analog of aminocaproic acid and has the same properties and used for the same indications. Like aminocaproic acid, concentrations needed to completely inhibit fibrinolysis in canine plasma after induction of hyperfibrinolysis were determined by Fletcher et al. (2014). It is available (Cyklokapron®, Lysteda®) as oral 500 mg tablets and 100 mg/L for IV infusion. Optimum doses have not been evaluated for animals, but in surgeries associated with bleeding in people, a dose of 100 mg/kg IV after induction of anesthesia has been used (Myles et al., 2017). The adverse effects of thrombosis formation is similar to the risks with aminocaproic acid. An additional adverse effect observed in people is an association between administration of tranexamic acid and an increased risk of neurological events, including seizures.

Another drug, aprotinin (Transylol®) is a kallikrein or protease enzyme inhibitor. It was removed from the market in 2007 because of concerns about safety.

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Section 8

Endocrine Pharmacology

26

Hypothalamic and Pituitary Hormones

Srujana Rayalam, Margarethe Hoenig, and Duncan C. Ferguson

Introduction

The hypothalamus and pituitary control the function of the thyroid, adrenal glands, and gonads. Neurons and endocrine gland cells share the characteristic of being able to secrete chemical mediators and being electrically excitable. Chemical messengers can be secreted as a neurotransmitter or as a hormone. The neuroendocrine systems consist of clusters of peptide- and monoamine-secreting cells in the anterior and middle portions of the ventral hypothalamus. Their fibers project via nerve fibers to terminals in the outer layer of the median eminence. The capillary plexus of the median eminence is proximate to the nerve terminals of the periventricular nucleus (PVN) hypothalamic neurons, which make corticotropin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), and somatostatin (growth hormone inhibitory hormone, GHIH); arcuate hypothalamic nucleus neurons make gonadotropic hormone–releasing hormone (GnRH), growth hormone–releasing hormone (GHRH), and the prolactin-inhibiting neurotransmitter dopamine. The concentrations of the releasing and inhibitory hormones in the median eminence are 10 to 100 times as great as in other parts of the hypothalamus because they are stored in the nerve terminals (Cone et al., 2003; Rijnberk, 1996). The process of neurosecretion is characteristic of the hypothalamic nuclei, which release hormones into the portal vessels mediating the release of the anterior pituitary hormones such as growth hormone (GH), prolactin (PRL), thyrotropin (TSH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), and the pro-opiomelanocortin (POMC)-derived peptides adrenocorticotropin hormone (ACTH), β-lipotropin (β-LPH), α-melanotropin (α-MSH), and the opioid β-endorphin (β-END). For each of the anterior lobe hormone systems (ACTH, LH, FSH, TSH, GH, and PRL), there is a closed-loop feedback system. Anterior lobe hormone and hypophysiotropic hormone secretions are suppressed by free (unbound) concentrations of hormones secreted by the respective end-organs, that is the thyroid, gonadal, and adrenal glands. Some hormones like PRL regulate their own secretion by inhibition via short-loop feedback on the hypothalamus. The somatotropes account for 50% or more of the anterior pituitary lobe cells, while other types of anterior lobe cells account for between 5 and 15% of the gland (Rijnberk, 1996). Table 26.1 outlines the location of neuroendocrine substances in the nervous system and endocrine organs. Tables 26.2, 26.3, and 26.4 show the key features of the structure and function of the hypothalamic regulatory and pituitary hormones. Many of these peptides find their clinical use as agents for diagnostic tests of pituitary or endocrine end-organ function. Therefore, the related hypothalamic and anterior pituitary hormones will be discussed concomitantly.

The magnocellular neurons in the supraoptic nucleus (SON) and PVN of the hypothalamus terminate in the posterior pituitary lobe and secrete vasopressin (antidiuretic hormone; ADH) and oxytocin into the circulation. Figure 26.1 outlines the hypothalamic pituitary endocrine system.

Anterior Pituitary and Associated Regulatory Hormones

The pituitary hormones can be classified into three general categories: ACTH-LPH, glycoproteins (LH, FSH, and TSH), and somatomammotropins (GH, PRL) (see Table 26.3).

Corticotropin and Related Peptides

Corticotropin-Releasing Hormone

Corticotropin-releasing hormone (CRH)-secreting neurons are found in the paraventricular nuclei, and their nerve endings terminate in external layers of the median eminence. CRH is synthesized as part of a prohormone of 196 amino acids in length and undergoes enzymatic modification to an amidated form of a 41-amino-acid peptide, which is identical in man, dog, rat, and horse

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Table 26.1 Location of hypothalamic and pituitary peptides. See text for explanation of abbreviations.

Substance	Neurotransmitter in nerve endings	Hormones secreted by neurons	Hormones secreted by endocrine cells
GnRH	+	+	+
TRH	+	+	
CRH	+	+	+
GHRH	+	+	+
Somatostatin	+	+	+
POMC derivatives	+		+
TSH	+		+
FSH	+		+
LH	+		+
GH	+		+
PRL	+		+
Oxytocin	+	+	+
Vasopressin	+	+	+

(Mol et al., 1994). CRH stimulates synthesis and secretion of POMC and ACTH by pituitary corticotrophs. Two major CRH receptor subtypes, CRH-R1 and CRH-R2, and a nonmembrane-associated CRH binding protein, have been identified (Hauger et al., 2003). Both the receptors belong to the G protein–coupled receptor superfamily, and are linked to adenylate cyclase. The CRH-R1 is the major subtype in the pituitary corticotroph, and it mediates the stimulatory actions of CRH on ACTH secretion through both the adenylate cyclase and calcium–calmodulin signal transduction systems (Klonoff and Karam, 1992; Tyrell et al., 1994). In addition, CRH and its receptors act outside the hypothalamic–pituitary–adrenal axis and maintain both acute and chronic synaptic stability to deal with daily or lifelong stressors (Orozco-Cabal et al., 2006). CRH neurons integrate inputs from physiological stressors such as osmotic challenge, hypoxia, and blood volume loss, and neurogenic stress such as fear and restraint. Chemical inputs to these neurons include inhibitory effects

Table 26.2 Hypothalamic regulatory hormones. See text for explanation of abbreviations.

Hormone	Action	Structure	Precursor	Target organ
TRH	Elevates TSH, PRL	Blocked tripeptide (pGlu-His-Pro-NH2)	29-kDa precursor containing five copies of TRH	Pituitary thyrotrope
GnRH	Elevates LH, FSH	Blocked decapeptide (pGlu…Gly-NH2)	Amino terminus of 90 amino acid precursor	Pituitary gonadotrope
Somatostatin	Decreases GH	14 Amino acid peptide with disulfide bond between residues 3 and 14	Carboxy- terminus of 92 amino acid precursor	Pituitary somatrotroph
CRH	Elevates ACTH	41 Amino acid peptide with amidated carbo xy-terminus	Carboxy- terminus of 190 amino acid precursor	Pituitary corticotroph
GHRH	Elevate GH	44 Amino acid peptide with amidated carbo xy terminus	Residues 32–75 from a 107 amino acid pre cursor	Pituitary somatotroph
Dopamine	Decreases PRL Increase GHRH or GH Decrease CRH or ACTH	Catechol		Pituitary lactotroph, somatotroph, corticotroph

Table 26.3 Anterior pituitary hormones. See text for explanation of abbreviations. *Source:* Adapted from Tyrell et al., 1994.

Hormone	Molecular weight	Amino acids	Other
I. ACTH-LPH			
ACTH	4,500	39	
β-LPH	11,200	91	Derived from POMC
β-END	4,000	31	
II. Glycoprotein			
LH	29,000	α subunit: 89 β subunit: 115	α subunits are identical within a species; β subunits contributes biological specificity
FSH	29,000	α subunit: 89 β subunit: 115	
TSH	28,000	α subunit: 89 β subunit: 112	
III. Somatomammotropins			
GH	21,500	191	Common ancestral hormone
PRL	21,500	198	

Table 26.4 Hormones of the hypothalamus and the pituitary gland. See text for explanation of abbreviations.

Hormone	Site of action (target organ)	Biologic activity
Hypothalamus		
Gonadotropin-releasing hormone	Anterior pituitary (AP)	Release LH and SH
Thyrotropin-releasing hormone	AP	Release TSH
Corticotrophin-releasing hormone	AP	Release ACTH
Somatotropin-releasing hormone	AP	Release somatotropin
Somatotropin-inhibitory hormone	AP	Inhibit somatotropin output
Prolactin-inhibitory hormone	AP	Inhibit prolactin output
Prolactin-releasing hormone	AP	Release prolactin
Adenohypophysis		
Par distalis (anterior lobe)		
Somatotropin (growth hormone)	General soma	Body growth (bone, muscle, organs), protein synthesis, carbohydrate metabolism, regulation of renal functions (glomerular filtration rate) and water metabolism; increases cell permeability to amino acids; favors lactation
Adenocorticotropin hormone (ACTH, corticotropin)	Adrenal cortex	Maintenance of structural integrity of adrenal cortex, regulation of glucocorticoid secretion by zona fasciculate
Thyroid-stimulating hormone (TSH, thyrotropin)	Thyroid	Maintenance of normal structure and function of the thyroid gland; production of thyroxin and analogs
Prolactin (lactogenic hormone)	Mammary gland	Possibly favors lactation

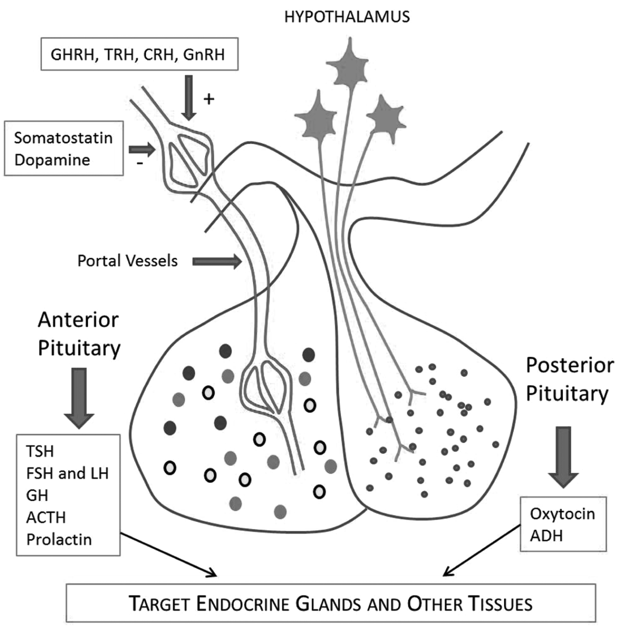

Figure 26.1 The hypothalamic pituitary endocrine system. Hypothalamic hormones regulate the release of anterior pituitary hormones. All the anterior pituitary hormones except for GH and prolactin act on their target endocrine glands to stimulate the production of hormones. GH has no single endocrine gland, instead it acts on liver and several other tissues. Prolactin and posterior pituitary hormones, ADH, and oxytocin act directly on target tissues like mammary gland, kidney, and uterus. ACTH, adrenocorticotropin hormone; ADH, antidiuretic hormones; CRH, corticotropin-releasing hormone, FSH, follicle stimulating hormones; GH, growth hormones, GHRH, growth hormone releasing hormone, GnRH, gonadotropin releasing hormone; LH, luteinizing hormone; TRH, thyrotropin releasing hormone, TSH, thyroid. stimulating hormone.

of γ-aminobutyric acid (GABA) and, of course, negative feedback from glucocorticoids. Stimulatory effects are provided by glutamate, serotonin, and cytokines such as interleukin (IL)-1, IL-2, IL-6, and tumor necrosis factor-α (TNF-α). Intravenous CRH, acting via CRH-R2β receptors on the cardiac atria and ventricles, results in hypotension and bradycardia (Cone et al., 2003). CRH has also been identified as an anxiogenic and anorexogenic factor, prompting research into CRH antagonists as both mood altering and appetite-stimulating drugs (Cone et al., 2003). A CRH inhibitory factor has been postulated, and atrial natriuretic peptide, activins, and inhibins are considered as candidate peptides (Engler et al., 1999).

Adrenocorticotropin

Biosynthesis

Adrenocorticotropin (ACTH) is a 39-amino-acid peptide hormone (MW 4,500), which is one of several products from the metabolism of the 267-amino-acid precursor molecule POMC (MW 28,500) (Figure 26.2). Between species, there is significant sequence homology in the ACTH amino acid structure. Canine ACTH differs by only one C-terminal amino acid from ACTH of other species (Mol et al., 1991). The other fragments of POMC with biological activity include β-lipotropin (β-LPH), α-melanocyte–stimulating hormone (α-MSH), β-MSH, and the opioid β–endorphin, as well as the N-terminal fragment (Figure 26.2). ACTH is metabolized to ACTH_{1-13}, which is identical to α-MSH, and corticotropin-like intermediate lobe

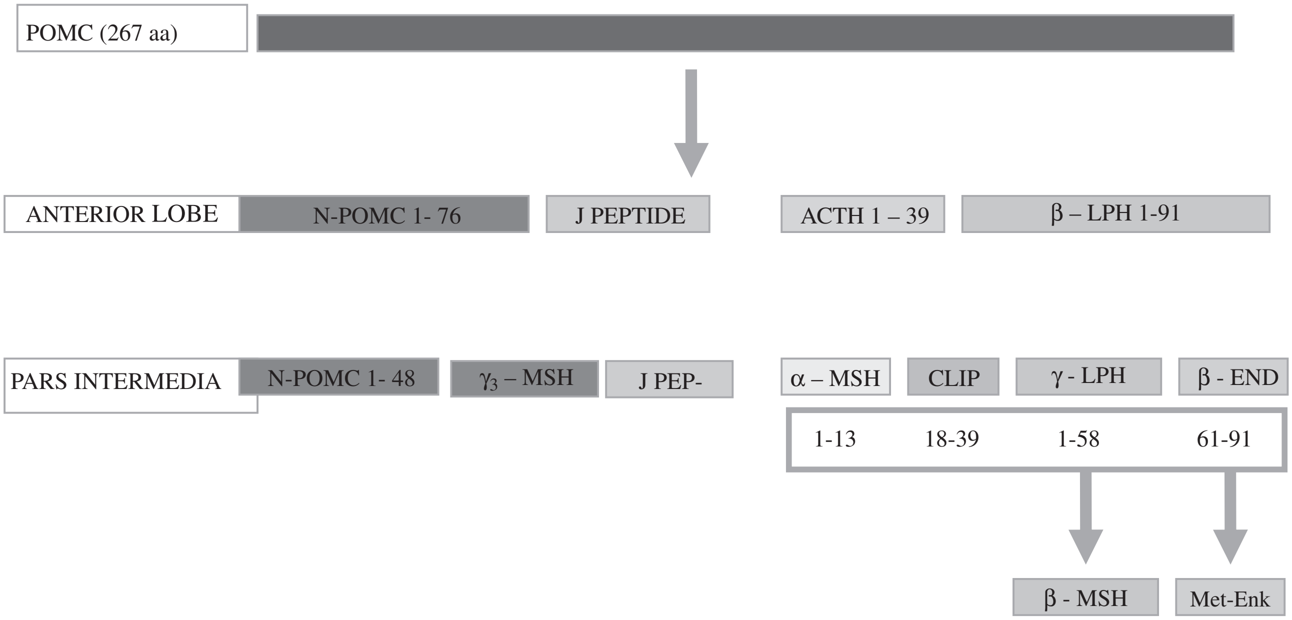

Figure 26.2 Relationships of pro-opiomelanocortin (POMC) derived peptides. Following synthesis in the anterior or intermediate lobe of the pituitary, POMC is cleaved to form adrenocorticotropin (ACTH) and β-lipotropin (β-LPH). ACTH further degrades, particularly in the intermediate lobe, to α-melanocyte stimulating factor (α-MSH) and corticotropin-like intermediate lobe peptide (CLIP). β-LPH is cleaved to produce γ-LPH and the endogenous opiate β-endorphin (β-END). γ-LPH is cleaved to produce β-MSH and β-endorphin is further processed to produce another opioid met-enkephalin (Met-Enk).

peptide (CLIP), which represents ACTH$_{18–39}$. These fragments are observed in species with developed intermediate lobes, such as the rat and horse, as well as fish, reptiles, and amphibians. β-LPH is secreted in equimolar amounts to ACTH. The 91 amino acids of β-LPH include the amino acid structure for β-MSH (41–58), γ-LPH (1–58), and β-END (61–91). The first (N-terminal) 23 amino acids of ACTH, which are identical in humans, cattle, pigs, and sheep, produce all of its biological effects (Klonoff and Karam, 1992; Tyrell et al., 1994). The sequence of the remaining amino acids varies among species (Chastain and Ganjam, 1986). MSH causes pigment granules in melanocytes to disperse so that skin will darken. Although genetic factors associated with skin color are more important in the higher vertebrates, in mammals, MSH may cause transiently increased pigment synthesis. In addition to the pigmentary action, α-MSH exhibits cytoprotective activity against UV-induced apoptosis and DNA damage in cutaneous tissue (Böhm et al., 2006).

Structure

ACTH is a peptide that contains 39 amino acids in a straight chain molecule in sheep, pig, cow, and human. The first 24 and last seven amino acids are identical in these species and there are minor differences in amino acids 25 through 32 (Figure 26.3). The amino acid sequence of canine β-END differs from the human sequence by four amino acids (Young and Kemppainen, 1994). The distribution of molecular forms of β-END in the canine intermediate lobe and anterior pituitary resembles most closely that in rats. In other species, such as sheep or horse, acetylated and shortened forms exist in substantial amounts. In all species studied to date, ACTH and related peptides are synthesized and cleaved from a common precursor molecule POMC. Posttranslational processing of POMC differs in the pars distalis and pars intermedia of the pituitary gland. In the pars distalis, POMC is processed to form ACTH, β-LPH, and some γ-LPH and β-END. In the pars intermedia, however, POMC is processed to ACTH and β-LPH; ACTH is then

1	2	3	4	5	6	7	8	9	10	11	12	13	--------	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Trp	Gly	Lys	Pro	Val	(human)	Asp	Ala	Gly	Glu	Asp	Gln	Ser	Ala	Glu	Ala	Phe	Pro	Leu	Glu	Phe
Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	Trp	Gly	Lys	Pro	Val	(pig)	Asp	Gly	Ala	Glu	Asp	Gln	Leu	Ala	Glu						
Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	TRp	Gly	Lys	Pro	Val	(beef)	Asp	Gly	Glu	Ala	Glu	Asp	Ser	Ala	Gln						
Ser	Tyr	Ser	Met	Glu	His	Phe	Arg	TRp	Gly	Lys	Pro	Val	(sheep)	Ala	Gly	Glu	Asp	Asp	Glu	Ala	Ser	Glu						

Figure 26.3 Amino acid sequences of human, pig, beef, and sheep adrenocorticotropin hormone (ACTH). Amino acids 1–13 are also present in α-melanocyte stimulating factor (α-MSH). Note areas of species homology in shaded boxes. *Source:* Klonoff and Karam, 1992.

further processed to α-MSH and CLIP, and β-LPH is further processed to β-MSH, β-END, and β-END metabolites. As a result, ACTH and β-LPH are intermediates to α-MSH and the opiate β-END. The pattern of POMC-derived peptide secretion from the pars intermedia has been characterized in rats, horses, pigs, sheep, dogs, and cats. The plasma POMC peptide concentrations found in cats is similar to that in rats, but is markedly different from that in dogs, in which the secretion of POMC peptides in the pars intermedia is normally low (Peterson et al., 1994b). N-POMC (1–77) promotes adrenocortical cell replication, and the growth-promoting actions may be a result of either direct action of N-POMC (1–77) at the fetal adrenal gland or a consequence of proteolytic cleavage of N-POMC (1–77) at the adrenal gland (Coulter et al., 2002).

Regulation of Secretion

β-LPH and β-endorphin are secreted in a pattern similar to ACTH, increasing in response to stress and paralleling ACTH in a variety of disease conditions. The regulation of ACTH is most directly influenced by the hypothalamic hormone CRH, which stimulates ACTH in a pulsatile fashion. Arginine-vasopressin (ADH) is also a potent stimulus for ACTH secretion (van Wijk et al., 1994). The pulsatility of ACTH release appears to occur in most species. Although a diurnal variation of cortisol exists in man, studies with sampling at 30-minute intervals for 48 hours have not confirmed a diurnal variation in ACTH in dogs or cats (Peterson et al., 1994c).

Four mechanisms have been identified to regulate ACTH secretion: (i) episodic secretion and possible diurnal variation, mediated by the CNS and hypothalamus; (ii) response to stress (cat much more sensitive than dog) mediated by the CNS and hypothalamus; (iii) feedback inhibition by cortisol at both the hypothalamus and pituitary; and (iv) immunological factors (IL-1, IL-6, TNF, etc.), which act at the hypothalamus to increase CRH (Rijnberk, 1996; Cone et al., 2003). Facilitatory and inhibitory pathways involving GABAergic, cholinergic, adrenergic, dopaminergic, and serotoninergic systems are all involved in hypothalamic regulation of ACTH and cortisol secretion. Drugs manipulating these systems have been utilized to manage pituitary-dependent hyperadrenocorticism in the dog and horse. Ergot alkaloids (dopamine agonists) and serotonin antagonists (cyproheptadine) have been utilized to manipulate ACTH release without much clinical success. The monoamine oxidase B inhibitor L-deprenyl was developed and approved by the FDA for use in dogs with pituitary-dependent hyperadrenocorticism (Bruyette et al., 1997a, 1997b). In theory, reduction of the rate of dopamine degradation by this class of drugs should increase dopamine and suppress ACTH secretion (Klonoff and Karam, 1992). Subsequent studies have been less enthusiastic about this drug as the sole treatment for canine pituitary-dependent hyperadrenocorticism (Reusch et al., 1999; Braddock et al., 2004).

Many factors stimulate ACTH secretion: pain, trauma, hypoxia, hypoglycemia, surgery, cold, pyrogens, and vasopressin (at least in man). CRH is often used pharmacologically to release ACTH from the pars distalis, and the drug haloperidol can be used to stimulate pars intermedia secretion with pars distalis effects being removed by dexamethasone. In cultured canine anterior pituitary cells, it appears that CRH stimulates ACTH secretion, but arginine vasopressin, oxytocin, and angiotensin II do not (Kemppainen et al., 1992).

Negative-Feedback Systems

Exogenous corticosteroids suppress the ACTH response to stress. Negative feedback of cortisol occurs via both the hypothalamic and pituitary. Feedback occurs in three ways:

1) Fast feedback is sensitive to the rate of change in cortisol and probably occurs via a nonnuclear receptor.
2) Slow feedback that is sensitive to the cortisol concentration in plasma. This feedback loop is tested by the low-dose dexamethasone suppression test.
3) Short-loop feedback by ACTH on the release of CRH by neurons in the hypothalamus and corticotrope receptors in the corticotropic cells of the anterior pituitary. The feedback is mediated through a type I mineralocorticoid-preferring receptor (MR) and a type II glucocorticoid-preferring receptor (GR). The highest level of GR in the dog brain is found in the septohippocampal complex and the anterior lobe of the pituitary (Reul et al., 1990; Keller-Wood, 1990; Klonoff and Karam, 1992; Tyrell et al., 1994).

Function

ACTH stimulates the secretion of glucocorticoids, mineralocorticoids, and adrenal androgens by increasing the activity of cholesterol desmolase, the enzyme that is rate limiting for steroid production and converts cholesterol to pregnenolone. ACTH also stimulates adrenal hypertrophy and hyperplasia. Steroids are not stored in the adrenal cortex, but they are immediately released upon stimulation of the zona fasciculata. ACTH causes growth of both the zona fasciculata and glomerulosa. The biological activity is conveyed by the amino terminal end of the molecule. ACTH stimulates adrenocortical growth and steroidogenesis by increasing cellular cAMP (Tyrell et al., 1994).

LPH induces lipolysis in adipocytes of some species, but its function is largely unknown other than serving as a precursor peptide for β-END, which is an endogenous opiate (Klonoff and Karam, 1992; Kuret and

Table 26.5 Dosage protocols for corticotropin-releasing hormone (CRH) stimulation test. *Source:* Crager et al., 1994; Moore and Hoenig, 1992; Peterson et al., 1994b, 1994c; Penninsula Laboratories.

Preparation	Horses /cattle	Dogs	Cats
Ovine CRH (μg/kg IV)	NA	1	1
Sampling times (hours)	NA	0, 0.5	0, 0.5

NA, not applicable.

Murad, 1990). However, a comparative study of the central effects of specific POMC-derived melanocortin peptides on food intake and body weight indicated that most of the POMC-derived melanopeptides, including γ-LPH, reduced food intake in POMC-null mice (Tung et al., 2006).

Diagnostic Uses of CRH and ACTH

The most important applications of CRH and ACTH are as a diagnostic agent to test adrenocorticotroph and adrenal functional reserve.

CRH Stimulation Test

The CRH stimulation test is used mainly as a research tool to assess pituitary ACTH secretory capacity. In animals administered exogenous glucocorticoids, the ACTH and cortisol response to CRH is diminished. In dogs and cats, ovine CRH is administered at 1 μg/kg intravenously and plasma is sampled for ACTH measurement at 0 and 0.5 hours for peak effect (Table 26.5). Other sampling times have been employed in research reports (Crager et al., 1994; Moore and Hoenig, 1992; Peterson et al., 1994b, 1994c; Scott-Moncrieff et al., 2003). Dogs with primary adrenal insufficiency have high basal plasma immunoreactive ACTH concentrations and show exaggerated responses to CRH, whereas dogs with secondary adrenal insufficiency showed undetectable basal plasma concentrations of immunoreactive ACTH that did not increase after stimulation with CRH (Peterson et al., 1992). On the other hand, studies of normal dogs and dogs with pituitary-dependent hyperadrenocorticism have shown that ACTH secretion is less sensitive to CRH than with lysine vasopressin (LVP). It was found that adrenocortical tumors develop an aberrant sensitivity to LVP, with adrenal tissue appearing to directly respond to LVP (van Wijk et al., 1994).

Preparations of ACTH

Synthetic human $ACTH_{1-24}$ is called cosyntropin (Cortrosyn®, Amphastar Pharmaceuticals). Repositol ACTH gel from animal (porcine) sources is no longer available commercially. However, when comparing dosage protocols, 1 unit of porcine ACTH is approximately equal to 10 μg of cosyntropin. Synthetic ACTH is well absorbed by the IM route. The biological half-life of all forms of ACTH is 10 to 20 minutes and has an effect on the adrenal cortex for 12 to 48 hours. Generic cosyntropin is also available from Sandoz, Inc and a study has indicated that injection of generic cosyntropin stimulates adrenocortical secretion to an equivalent degree, as compared with Cortrosyn in dogs (Cohen and Feldman, 2012).

Table 26.6 Dosage protocols for adrenocorticotropic hormone (ACTH) stimulation test. *Source:* Hansen et al., 1994; Moore and Hoenig, 1992; Peterson et al., 1994b, 1994c.

Preparation	Horses /cattle	Dogs	Cats
ACTH, USP			
Dosage IV, IM, SC (IU/kg)	1	2.2	2.2
Sampling times (hours)	0, 8	0, 2	0, 1, 2
Synthetic cosyntropin (Cortrosyn, Organon Pharmaceuticals)			
Dosage IM, IV (μg)	NA	250	125 (IV preferred)
Sampling times (minutes)	NA	0, 1	0, 1

NA, not applicable or available.

ACTH Stimulation Test

The main use of ACTH is the differential diagnosis of adrenocortical hyperplasia from adrenocortical neoplasia (primary) in dogs, cats, and horses (Table 26.6), and in the definitive diagnosis of primary adrenal hypofunction. ACTH is well absorbed following IM injection. Following injection of aqueous synthetic ACTH, largely because of the short half-life of ACTH, plasma cortisol concentrations peak at 30 to 90 minutes. Therefore, most sampling protocols with aqueous ACTH in dogs recommend sampling times at 1 hour after administration for a peak effect. The administration of 250 μg synthetic ACTH to dogs resulted in similar cortisol patterns, whether the dose was given IV or IM, despite the fact that there were much higher peak ACTH concentrations with the intravenous dose. Additional studies further confirmed that in healthy dogs and dogs with hyperadrenocorticism, administration of cosyntropin, a synthetic derivative of ACTH, at a dose of 5 μg/kg, IV or IM, not only resulted in equivalent adrenal gland stimulation (Behrend et al., 2006) but is also effective for diagnosing hypoadrenocorticism in dogs (Lathan et al., 2008). The peak cortisol concentration was at 60–90 minutes (Hansen et al., 1994). Interestingly, the increase in cortisol concentrations from 1–2 hours post-ACTH injection at a dose of 250 μg/animal IV in adult Cocker Spaniel dogs, was significantly higher in females than males (Pessina et al., 2009). However, in cats, the intravenous dose of synthetic ACTH appeared to provide a greater response compared to the IM dosage but the peak cortisol response is somewhat variable and post-ACTH samples at 45 and 60 minutes are recommended (Peterson et al., 1994b, 1994c).

Evaluation of Partial or Relative Adrenal Insufficiency in Foals

Studies have sought to evaluate the dose-dependency of the cortisol response to ACTH as an index of adrenal insufficiency in systemically ill foals. A randomized crossover study of intravenous ACTH (Cosyntropin) in healthy foals at 3–4 days of age demonstrated that while 1 μg of ACTH had not yet reached a threshold dose, 10 μg resulted in a peak cortisol at 30 minutes returning to baseline by 90 minutes, while 100 and 250 μg both elicited a peak cortisol response at 90 minutes (Hart et al., 2007). In contrast, administration of ACTH (Cosyntropin) at a dose of 0.1 μg/kg resulted in maximum adrenal stimulation, with peak cortisol concentration 30 minutes after ACTH administration in healthy adult horses.

Melatonin

The pineal gland consists of photoreceptive cells, which have functional significance in fish and amphibians, but the pinealocytes function mainly as secretory cells in higher vertebrates. In vertebrates, light–dark cycle information is related to the pineal via indirect multisynaptic input, crucial to the control of biological rhythmicity. The retina provides light information to the suprachiasmatic nucleus (SCN) of the hypothalamus via the retinohypothalamic tract. The SCN provides input to the PVN via intrahypothalamic projections. The PVN then innervates sympathetic preganglionic neurons in the intermediolateral neurons in the cranial cervical ganglion, which then provides noradrenergic input to the pineal gland.

In mammals, the pinealocytes are the source of melatonin. Melatonin is synthesized from the amino acid tryptophan, via serotonin. Melatonin synthesis is tightly controlled in a circadian manner and is entrained to the light–dark cycle with highest melatonin concentrations during dark periods. The signal transduction associated with these changes in melatonin secretion is as follows: the absence of light leads to norepinephrine release from sympathetic nerve terminals that act upon G-protein coupled β-adrenergic receptors, which increase adenylate cyclase activity. Cyclic AMP–response element in the promoter region for *N*-acetyltransferase is the rate-limiting step for melatonin synthesis. Melatonin then acts on a family of G-protein coupled receptors (Mel1a, Mel1b, and Mel1c), depending upon the species of animal. Melatonin thereby inhibits the activity of neurons in the SCN, considered the circadian pacemaker of the mammalian brain. This regulation is key to entraining reproductive patterns in species such as the sheep and horse.

Melatonin can be used as therapy for alopecia X in dogs. Alopecia X is a controversially defined condition (or conditions) associated with hair loss in dogs, which cannot be attributed to known endocrinopathies such as hypothyroidism and hyperadrenocorticism. One proposed treatment is melatonin at a dose of 3–6 mg/dog once or twice a day (Frank et al., 2004). Response rate may vary greatly depending on the severity and chronicity of the problem, and recurrence is not uncommon despite therapy.

Glycoprotein Hormones and Associated Releasing Hormones

Only the thyroid-related peptides thyrotropin-releasing hormone (TRH) and thyroid stimulating hormone (TSH) will be discussed in this chapter. Gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), placental chorionic gonadotropin (HCG), and pregnant mare serum gonatropin (PMSG) are discussed in Chapter 27 on reproductive drugs.

Thyrotropin-Releasing Hormone

Thyrotropin-releasing hormone (TRH) is a tripeptide: PyroGlu-His-Pro-NH_2. Neurons secreting TRH are located in the medial portion of the paraventricular nuclei and their axons terminate in medial portions of the external lamina of medial eminence. TRH is present extensively in the brain outside of the classic "thyrotropic area" of the hypothalamus. An intact amide and the cyclized glutamic acid terminus are essential for activity. TRH is synthesized as a large 242-amino-acid precursor, which contains five repeating sequences in the rat and six in the human called *preprothyrotropin-releasing hormone* (preproTRH) (Jackson, 1982; Lechan et al., 1984; Yamada et al., 1990). The prohormone undergoes extensive posttranslational processing, including enzymatic cleavage, cyclization of NH_2-terminal glutamic acid, and exchange of an amide for the COOH-terminal glycine. TRH binds to specific receptors on the plasma membrane of the pituitary cell (Halpern and Hinkle, 1981). TRH activates membrane adenylate cyclase with the formation of cAMP and the increase in cAMP stimulates TSH secretion. However, cAMP may not increase under all conditions of TRH-induced TSH release, and increased intracellular cAMP is not always associated with an increase in TSH secretion. There is growing evidence that the TRH-induced increase in cAMP concentrations may be a secondary event. It is now widely accepted that TRH action is mediated mainly through activation of phospholipase C. It causes hydrolysis of phosphatidyl-inositol-4,5-biphosphate (PIP_2) into inositol-1,4,5-triphosphate (IP_3) and 1,2-diacylglycerol (DAG). DAG then activates protein kinase C. Continuous exposure of TRH receptors to TRH causes an increase in

IP_3 concentration that peaks within 10 seconds and falls within 1 minute, indicating the rapid desensitization of the TRH response (Yu and Hinkle, 1997). The binding of β-arrestins is shown to be critical for desensitization (Jones and Hinkle, 2005). TRH induces an immediate and rapid increase in intracellular free calcium, which decays rapidly, followed by an extended plateau of elevated calcium. The first phase reflects increased release of intracellular calcium stores, whereas the second phase represents calcium influx. This biphasic action correlates with electrical charges, induction of Ca^{2+} fluxes, and secretory activity in pituitary cells (Geras and Gershengorn, 1981; Gershengorn et al., 1980; Tashjian et al., 1987; Vale et al., 1977; Winiger and Schlegel, 1988).

TRH Stimulation Test

Diagnosing hypothyroidism in the dog: The TRH stimulation test, as it is used in the diagnosis of human pituitary and thyroid disease, is designed to evaluate the pituitary's responsiveness to TRH as manifested by the change in serum TSH concentration. In primary thyroid gland failure, the pituitary response to TRH is increased, and, in hyperthyroidism, it is decreased.

TRH has been most commonly applied instead of exogenous TSH to evaluate thyroid functional reserve by the increment in serum thyroxine (T_4) concentrations. Figure 26.4 shows the theoretical response of serum T_4 to TRH and TSH administration. In theory, the administration of TRH should lead to an increase in T_4 only if the pituitary–thyroid axis is intact (Figure 26.4). Therefore, responsiveness to TRH should be observed only in tertiary (hypothalamic) thyroid insufficiency, a condition not yet documented in the dog (Ferguson, 1984, 1994, 2007). Since the availability of the canine TSH assay in 1997, more has been learned about regulation of TSH secretion. In human patients, an increased response of TSH to TRH generally demonstrated an enhanced response in early or mild primary hypothyroidism. However, evaluation of TRH-stimulated TSH responsiveness has not proved to be of much more diagnostic value than baseline canine TSH concentrations (Scott-Moncrieff et al., 1998; Hoenig and Ferguson, 1997; Ferguson, 2007). Furthermore, chronic hypothyroidism

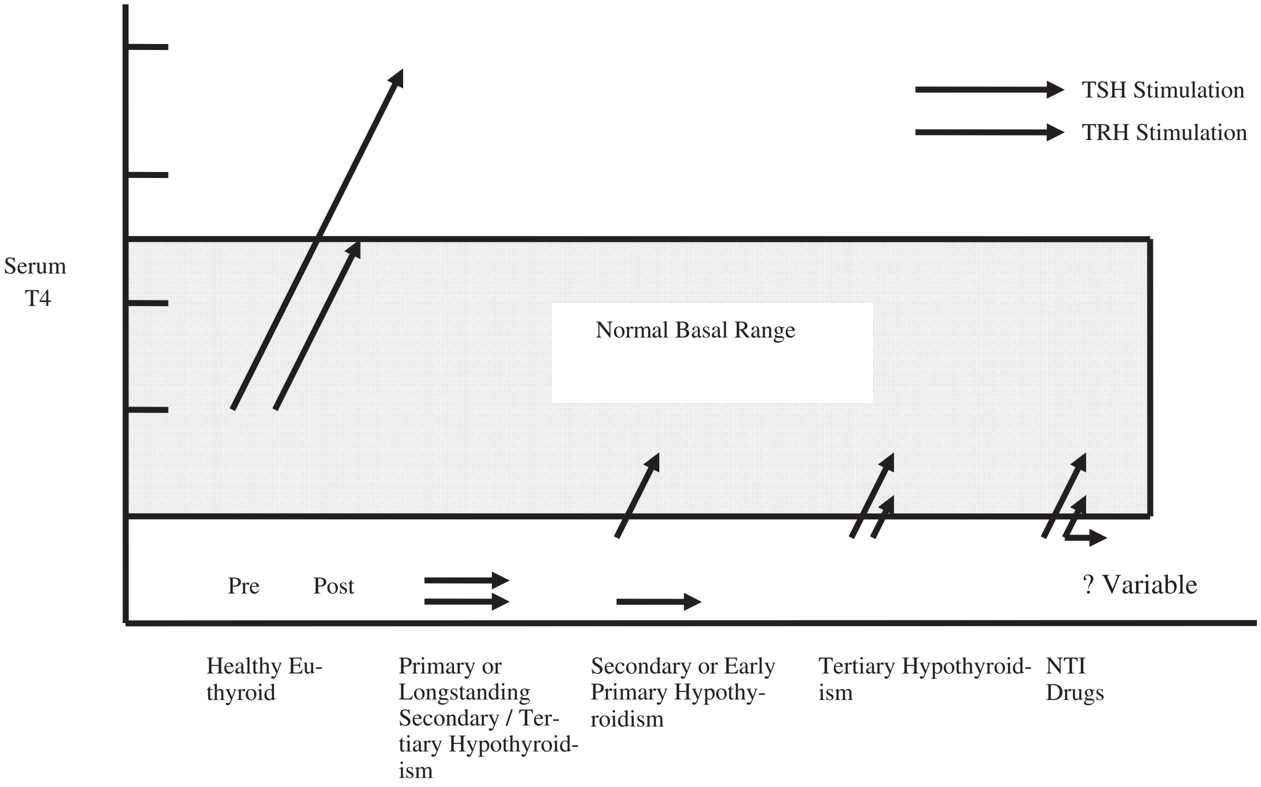

Figure 26.4 Diagnostic classifications based upon thyrotropin-releasing hormone (TRH) and thyrotropin (TSH) stimulation tests. The arrows represent in a stylized fashion the classical response of serum thyroxine (T_4) concentrations to optimal doses of TRH and TSH. The actual magnitude of response will, in part, be determined by the dosage of TRH and TSH and the time of serum sampling post-TRH or post-TSH. The shaded area represents the normal baseline serum T_4 concentrations. Note the lower relative response to even maximal dosages of TRH compared to TSH. The left arrow in each pair of arrows (darker arrow) represents the response to TSH and the right arrow (lighter) represents the response to TRH. NTI, nonthyroidal illness. *Source:* Peterson and Ferguson, 1989.

Table 26.7 Protocols for TRH stimulation test. *Sources:* Ferguson, 1984, 1994; Lothrop and Nolan, 1986; Lothrop et al., 1984; Peterson and Ferguson, 1989; Peterson et al., 1994a, 1994c.

	Dose	Route	Sampling times (hour)
Dogs	200 µg	IV	0, 4
Cats	100 µg/kg	IV	0, 4
Horse	1000 µg	IV	0, 4

Preparations used are Relefact TRH® (Hoechst-Roussel); Thypinone® (Abbott Diagnostic); TRH (Peninsula Laboratories).

has been shown to be associated with a rise then a fall in baseline TSH. This observation may contribute to as much as 25–40% of dogs with primary hypothyroidism having a normal, and not elevated, TSH concentration. It is not yet clear whether this phenomenon is associated with a desensitization toward negative feedback; however, TRH-stimulated increments in TSH seem to decline with time (Diaz-Espineira et al., 2008).

A variety of TRH dosages have been proposed for use in the dog. In general, regardless of the species, increasing the TRH or TSH dose increases the duration of the serum T_4 response. In the dog, side effects were more significant at dosages greater than 100 µg/kg; salivation, urination, defecation, vomition, miosis, tachycardia, and tachypnea were observed (Table 26.7). The recommended protocol for the TRH stimulation test is the administration of 100 µg/kg TRH intravenously with the collection of serum for T_4 measurement at 0 and 6 hours post-TRH if serum T_4 is measured, and at 0 and 30 minutes if serum TSH is measured. Using this protocol in normal dogs, at least a 50% increase in serum T_4 was observed in 90% of dogs and all dogs had an increase of at least 0.5 µg/dl (6.4 nmol/l) above baseline (Figure 26.4). With the lower dose of 200 µg TRH per dog, serum T_4 was shown to be maximal 4 hours after TRH administration. Because of the small increments following TRH, the successful application of the TRH stimulation test requires the use of a T_4 assay with extremely good internal reproducibility or preferentially, measurement of canine TSH.

Diagnosing hypothyroidism in the horse: TRH has been evaluated in horses as an alternative for establishing the pituitary thyrotrope and thyroid functional reserve. Intravenous administration of 1,000 µg of TRH increases serum concentrations of T_4 and T_3 peaking at 4 hours post-TRH (Table 26.7) (Lothrop and Nolan, 1986). TRH has also been used at a dose of 2,000 µg IV with samples at 0, 30, and 90 minutes, to evaluate possible pituitary disease in the horse. However, there was no increase in progesterone, 17-hydroxyprogesterone, androstenedione, cortisol, aldosterone, testosterone, or estradiol, raising the question about the validity of TRH for evaluation of adrenal dysfunction in the horse (Fecteau et al., 2005).

Diagnosing mild hyperthyroidism in the cat: In some instances, thyroid hormone concentrations (T_4 and T_3) are normal, are borderline, or even fluctuate into and out of the normal range in cats with mild hyperthyroidism. The TRH stimulation has been studied as a test of thyroid autonomy. In euthyroidism, the thyroid gland of the cat responds significantly to intravenous TRH, but the response in the hyperthyroid cat is considerably less, implying that thyroid function is not under the influence of endogenous TSH. The dosage of 100 µg/kg TRH administered intravenously is followed by serum sampling at 4 hours after injection. Serum T_4 concentrations increased by >50% in all normal cats and cats with nonthyroidal disease, whereas only 11% of hyperthyroid cats showed a 50% increase in serum T_4 concentration after TRH administration. Although case-controlled studies have established relative specificity, others have questioned the specificity the TRH stimulation test results in the presence of nonthyroidal illness (Peterson et al., 1994a, 2001). Adverse side effects in the cat associated with administration of TRH were common and included transient vomiting, salivation, tachypnea, and defecation. As a diagnostic test, the TRH stimulation test compares favorably with the T_3 suppression test while requiring less time, and it is more convenient to perform (Peterson et al., 1994a, 1994c). The biological activity of freshly reconstituted TRH is maintained when frozen at −20°C for 1 to 5 weeks (Rosychuk et al., 1988).

Thyrotropin

Chemistry

Thyroid stimulating hormone (TSH) is a glycoprotein of MW 28,000 that is synthesized by thyrotrope cells of the anterior pituitary (Table 26.3). It is chemically related to the family of glycoproteins, including the pituitary gonadotropins LH, FSH, and placental chorionic gonadotropin (CG). Each of these hormones contains two noncovalently linked dissimilar polypeptide chains, alpha and beta.

LH, FSH, and TSH all share similarities in tertiary structure conferred by the evolutionary preservation of cysteine residues, particularly those involved in the “cysteine knot” motif identified by crystallographic information. Furthermore, sites of *N*-glycosylation also appear to be preserved during evolution. As described below, glycoproteins contribute to the tertiary structure as well as the functional and immunogenic characteristics of the dimers (Zerfaoui and Ronin, 1996).

Alpha and Beta Subunits

TSH is a heterodimer composed of two noncovalently bound subunits, alpha and beta. The subunits are synthesized as separate peptides from distinct mRNAs (Vamvakopoulos and Kourides, 1979). The alpha subunit is common to all three hormones, but the beta subunit is unique for each hormone and confers biological specificity. For all four glycoproteins within each species, the amino acid sequence of the alpha subunit is common. However, the carbohydrate structure may vary. Microheterogeneity of the carbohydrate constituents of the individual hormones causes heterogeneity in receptor affinity, biological potency, and metabolic clearance (Pierce and Parson, 1981; Wondisford et al., 1988).

The alpha subunits are approximately 20 to 22 kD, have 92 to 96 amino acid residues, and contain two N-linked carbohydrate groups. The human, cow, mouse, dog, cat, and rat alpha subunit genes are similar (Yang et al., 2000b; Rayalam et al., 2006a, 2006b). All species have a single mRNA species that is between 730 and 800 bases long. The mRNA encodes the precursor of the alpha subunit and a leader sequence of an average of 24 amino acids.

The TSH beta subunit is approximately 18 kD, and consists of approximately 110–113 amino acids and contains one N-linked complex carbohydrate (Green and Baenziger, 1988). TSH, like LH, contains sulfate groups that terminate certain chains; such sulfation is found only to a small extent in FSH and not at all in CG. The genes for beta subunit of TSH of mouse, cow, human, dog, and horse have been cloned and recombinant canine TSH (Yang et al., 2000a, 2000b) and feline TSH (Rayalam et al., 2006a, 2006b) have been expressed in vitro. Each mRNA is approximately 700 bases in length with minor variations. The TSH beta mRNA encodes the precursor TSH beta subunit with a 20–amino-acid leader sequence and a 117– or 118–amino-acid coding region.

The alpha subunit peptide is more abundant than the unique beta subunit of peptides. Free serum alpha subunits are secreted and are present in equivalent concentrations to combined concentration of TSH, LH, and FSH. Free serum beta subunit concentrations are lower, usually below the level of detectability. Overabundance of alpha subunit suggests that regulation of beta subunit synthesis is the rate-limiting step in modulating the levels of TSH, LH, and FSH (McNeilly et al., 2003; Taylor and Weintraub, 1985).

Action

TSH binds to specific receptors on the thyroid plasma membrane. TSH receptors (TSHR) from dog, cat, pig, human, and rat have been completely cloned and species comparisons reveal a high degree of homology of more than 90%. The extracellular domain of the receptor contains 398 amino acids with five sites for N-linked glycosylation. The intracellular domain has 346 amino acids with seven putative transmembrane segments. The stimulatory guanine nucleotide regulatory protein binds to the third intracellular loop. The receptor has a glycoprotein component and a ganglioside, which may be involved in TSH activation of adenylate cyclase. TSHR is unique among glycoprotein hormone receptors in that some mature receptors on the cell surface are cleaved into two subunits (Rapoport et al., 1998). Only intact TSH binds to the receptor and the beta subunit does not possess biological activity (Chan et al., 1987; Field, 1975). Binding of TSH to its receptor activates adenylate cyclase and subsequent accumulation of cAMP, but is not dependent on the interaction of the TSH from that species with the receptor. This results in the stimulation and dissociation of the regulatory and catalytic subunits of cAMP-dependent protein kinase (protein kinase A) with subsequent phosphorylation of various cellular proteins resulting in an increase in thyrocyte iodide uptake, thyroid hormone organification, and thyroid hormone secretion. This cyclic AMP–mediated pathway appears to be important in both thyroid hormone secretion and thyroid glandular growth (Chayoth et al., 1985). Another important function of TSH in this context would be the up-regulation of sodium iodide symporter (NIS) in the thyroid cells. Several in vivo and in vitro studies showed that TSH stimulates not only iodide transport activity but also NIS gene and protein expression (Kogai et al., 1997, 2000; Saito et al., 1997).

The binding of TSH is also known to activate the phospholipase C signaling system. Activation of phospholipase C results in hydrolysis of PIP_2 with formation of DAG and IP_3. The former activates a Ca^{2+}-phospholipid–dependent protein kinase (protein kinase C) and the latter increases intracellular Ca^{2+} concentrations. The effect of TSH on phospholipase C is slower and requires larger amounts of the hormone than its activation of adenylate cyclase, suggesting a high-capacity and low-affinity TSH binding site. The physiological significance of activation of PIP_2 hydrolysis by TSH is not known, but is suspected to be involved in thyroid glandular proliferation (Chayoth et al., 1985; Taguchi and Field, 1988).

Preparations

Two bovine thyrotropin products have been available in the past. Availability of biochemically purified native TSH from bovine pituitary glands as a pharmaceutical has been drastically reduced following the introduction of human recombinant TSH and following the emergence of bovine spongiform encephalopathy. In addition, significantly higher endotoxin levels were detected in commercially available bTSH products, discouraging their use to prevent side effects (Schaefer et al., 2013). Nonetheless, investigators occasionally still use cell culture grade bTSH product for animal use. Although canine and feline recombinant TSH could successfully be synthesized, it is not commercially available (Yang et al., 2000a; Rayalam et al., 2006a). Regardless of the source, it is stored in a lyophilized form for reconstitution and parenteral administration. A study evaluated the effects of freezing reconstituted bovine TSH and demonstrated that bioactivity remained intact for at least 3 weeks at 4°C (Bruyette et al., 1987; Ferguson, 1994). Furthermore, the effect of storage conditions on the use of recombinant human thyrotropin (rhTSH) for TSH stimulation testing in dogs indicated that reconstituted rhTSH can be stored at 4°C for 4 weeks and at −20°C for 8 weeks without loss of biological activity (De Roover et al., 2006).

Thyrotropin Stimulation Test

The administration of exogenous bovine or human TSH followed by the measurement of serum T_4 and/or T_3 is a procedure still used occasionally to confirm the diagnosis of hypothyroidism, or loss of thyroid secretory reserve. Historically, it has been applied primarily in the dog, cat, and horse. The availability of immunoassays measuring endogenous canine TSH have reduced the practicality and need for this test. However, the TSH stimulation test continues to be considered a noninvasive test for confirming the reduced thyroid functional reserve associated with hypothyroidism.

Protocols for this test in the dog vary widely and are summarized in Table 26.8. Although the TSH dose and serum sampling times are often dictated by practical and economic considerations, increasing the TSH dose administered generally delays the time of the T_4 peak, and, up to a limit, results in a higher serum T_4 response and a plateau that is maintained for a longer period of time. The route of administration may be intravenous, intramuscular, or subcutaneous; however, the most consistent and rapid response is seen after intravenous dosing. For the dog, the suggested protocol is to draw a baseline blood sample for serum T_4 determination, and then administer 0.1 IU/kg TSH intravenously (maximum dose 5 units), followed by a blood sample at 4 or 6 hours post-TSH (Ferguson, 1984, 1994, 2007). Studies using 1 unit of bovine TSH (bTSH) per dog have indicated that the mean increase in serum T_4 and T_3 above baseline at 6 hours post-TSH was significantly lower following TSH at 1 IU than TSH at 5 IU but was not significantly different at 4 hours post-TSH for the two doses of TSH evaluated. Based on the criteria for adequate response to TSH, TSH at 1 IU led to classification of 35% of the dogs as having a decreased response to TSH at 4 hours and 35% at 6 hours. TSH at 5 IU resulted in no dogs having a decreased response at 4 hours and 1 dog in 20 (5%) at 6 hours (Beale et al., 1990).

Table 26.8 Protocols for thyrotropin-releasing hormone (TSH) stimulation test. Sources: Beale et al., 1990; Chen and Li, 1987; Ferguson, 1984,1994; Peterson and Ferguson, 1989; Sauvé and Paradis, 2000; Stegeman et al., 2003.

	Dose or dosage	Route	Sampling times (hours)
Dog	0.1 unit/kg (max. 5 units)	IV	0, 4
	1 unit/dog	IV	0, 4
	50 μg/dog[a]	IV	0, 4, 6
Cat	1 unit/kg	IV	0, 6
	1 unit/cat	IV	0, 4
	25–200 μg/cat[a]	IV	0, 6
Horse	5–10 units	IV	0, 4, 6

Preparation used was bovine TSH (Thytropar®; Rhone-Poulenc Rorer).
[a] Preparation used was recombinant hTSH (Thytropar®, Genzyme Corporation).
For older protocols in the dog, see Ferguson, 1984 for a review.

Pituitary-source preparations of TSH have been standardized according to bioactivity, which is reported as International Units (IU). Highly purified TSH approaches a biological specific activity of 12.5 IU activity/mg protein. A study compared the biological activity of recombinant human TSH (rhTSH) with bTSH in dogs, where bTSH was administered (1 unit corresponding to 500 μg in 0.5 ml of sterile water) intramuscularly and rhTSH was administered (75 μg in 0.5 ml sterile water) both intramuscularly and intravenously. Blood samples were collected immediately before and 6 hours after TSH administration. There were no significant differences in post-TSH stimulation T_4 concentrations among the three groups (Boretti et al., 2006). Another study suggested that 50 μg rhTSH was the optimal dose in euthyroid dogs to get a significant increase in serum total thyroxine (TT_4) concentrations (Sauvé and Paradis, 2000). Similarly, IV administration of 25–200 μg rhTSH significantly increased serum TT_4 concentrations 6–8 hours later in euthyroid cats (Stegeman et al., 2003). A previous study with bovine TSH in cats had shown that the peak of serum T_4 was simply extended to later time points with higher dosages (Hoenig and Ferguson, 1983).

Assuming a pharmacological dosage of TSH is used, the diagnosis of hypothyroidism in the dog is usually confirmed when the post-TSH serum T_4 concentration is below the normal range for basal T_4 (usually <1.0 μg/dl or <13 nmol/l in the dog) and rarely increases greater than 0.2 μg/dl (2.6 nmol/l) above the baseline. In pituitary forms of hypothyroidism, the thyroid gland should remain responsive to TSH. The rare cases of long-standing secondary (pituitary) or tertiary (hypothalamic) hypothyroidism with subsequent thyroid atrophy may require two or three consecutive daily doses of TSH to eventually demonstrate thyroid responsiveness (Ferguson, 1984, 1994, 2007).

A study of normal dogs evaluated the predictive value of blood sampling times after 5 units of intravenous TSH: in 80% of the animals, a doubling of the serum T_4

concentration was not achieved by 4 hours but was achieved in all cases by 6 hours post-TSH. Animals that responded favorably to thyroid replacement therapy have, on average, virtually no increase in serum T_4 concentration following TSH. An advantage of the TSH stimulation test is that post-TSH T_4 concentrations tend to be less variable because the thyroid is maximally stimulated.

Thyrotropin: Structural Homology Among Species

The TSH beta subunit is approximately 18 kD in molecular weight, consists of approximately 110–113 amino acids, and contains one *N*-linked complex carbohydrate. TSH, like LH, contains sulfate groups that terminate certain chains; such sulfation is found only to a small extent in FSH and not at all in CG. The genes for the beta subunit of TSH of mouse, rat, human, cattle, sheep, dog, cat, horse, opossum, teleost fish, chicken, quail, and horse have been cloned and sequenced. For most mammalian species, the mRNA is approximately 700 bases in length with minor variations. The TSH beta mRNA encodes the precursor TSH beta subunit with a 20-amino-acid leader sequence and a 117– or 118–amino-acid coding region. There is 80 to 90% homology at the amino-acid level among the sequences of most of the mammalian TSH beta molecules. As an example of avian species, the amino acid sequence of the quail TSH beta subunit shows homologies of about 70% to that of mammalian species, about 60% to that of amphibian, and about 50% to that of teleost fish. There is evidence that the functional domains of the TSH beta subunit and the TSH receptor have diverged cooperatively during evolution. Many regions of identical sequences are apparent in various beta subunits of glycoprotein hormones and the regions around residues 51 through 57 and 75 through 80 in the beta structures are suggested to be involved in interaction with the common alpha subunit (Szkudlinski et al., 2002).

Glycosylation Patterns: Relevance to Bioactivity and Immunoreactivity

The pituitary glycoproteins LH, FSH, and TSH are produced and secreted in multiple molecular forms. In vivo, microheterogeneity of the carbohydrate constituents of the individual hormones causes heterogeneity in affinity for the receptor and in metabolic clearance of the hormone. In vitro, immunoreactivity is affected by this heterogeneity. Much has been learned from studies of human and equine chorionic gonadotropin because of the use of these agents as pharmaceuticals. For example, highly sialylated human FSH variants exhibit lower receptor binding, bioactivity, and immunoactivity compared to less sialylated counterparts. Each isoform appears to have a different affinity for the receptor. For example, FSH glycosylation variants appear to induce or stabilize distinct receptor conformations, resulting in different degrees of activation or inhibition of a given signal transduction pathway. Moreover, the FSH microheterogeneity depends on not only the variations in the sialic acid content but also on the differences in the internal structure of the carbohydrate chains, which determine the full biological expression of FSH glycosylation variants (Creus et al., 2001).

The oligosaccharide chains of pituitary glycoprotein hormones such as human thyroid-stimulating hormone (hTSH) have been shown to be important in biosynthesis, subunit association, secretion, and bioactivity. However, the exact biological significance of these glycosylation isoforms remains controversial. hTSH glycosylation variants more basic in isoelectric point were found to be significantly more active than acidic ones in stimulating cAMP formation; however, there were no differences in stimulation of inositol phosphate release. In support of efforts to develop recombinant pituitary glycoproteins as pharmaceuticals, the bioactivity of glycoproteins produced in vitro appears to be as great as, or in some cases greater than, that of the pituitary derived form (Thotakura et al., 1991). Since glycosylation of plasma hormones is immunologically distinct from pituitary stock (Zerfaoui and Ronin, 1996), an ideal immunoassay standard's recognition should be glycosylation independent. In a study comparing the immunoreactivity and bioactivity of recombinant feline TSH with deglycosylation of recombinant feline TSH, deglycosylation appears not to have reduced the in vitro bioactivity of this product (Rayalam et al., 2006b). No studies have evaluated the rate of clearance of recombinant TSH in domestic species.

Effects of Drugs on TSH Concentrations

This subject has been reviewed (Daminet and Ferguson, 2003; Ferguson, 2007). Drugs with significant effects on the thyroid axis include glucocorticoids and barbiturates. However, suppression of TSH concentrations by glucocorticoids have not been observed, probably due to the lack of sensitivity of the TSH assay.

Two studies (Daminet et al., 1999; Gaskill et al., 1999) have examined the effects of phenobarbital on thyroid function tests. Daminet and coworkers prospectively examined the effect of a 3-week course of phenobarbital on TT_4, free thyroxine by equilibrium dialysis (FT_4D), and TSH, and observed no significant alteration of these values over this time frame. Gaskill and coworkers, in an effort to dissect out the effects of phenobarbital, documented TT_4 and TSH in 78 dogs receiving phenobarbital and compared them with 48 untreated epileptic dogs. Of the dogs on phenobarbital, 40% had low TT_4 and 7% had elevated TSH, while only 8% of untreated dogs had low TT_4 and none had elevated TSH. Of the latter group, only dogs with recent seizure activity had low TT_4 values. The investigators found no effect of phenobarbital on serum binding of T_4. The mean serum TT_4 was significantly lower, and mean serum TSH significantly higher in the phenobarbital-treated group. As with the subacute study (Daminet et al., 1999), there did not appear to be a correlation between phenobarbital dosage or duration of treatment and the serum TT_4 and TSH concentrations.

Dopamine and dopamine agonist bromocryptine were shown to suppress serum TSH in patients with selective pituitary resistance to thyroid hormone (Ohzeki et al., 1993). Other drugs that suppress TSH either at the level of hypothalamus or pituitary include retinoids (vitamin A derivatives) and somatostatin analogues (reviewed by Haugen, 2009).

Somatomammotropins and Regulatory Hormones

Growth hormone and prolactin have similarities in amino acid structure and also share some biological activities. As a result they are called somatomammotropic or somatolactotropic hormones.

Growth Hormone–Releasing Hormone

Growth Hormone–Releasing Hormone (GHRH) is secreted by neurons located in the arcuate nuclei with their axons terminating in external lamina of median eminence. GHRH is synthesized from a precursor of 108 amino acids. Initially, two peptides of 44 and 40 amino acids with GH-releasing activity were isolated. Synthetic peptides that contain only the first 29 amino acid residues of GHRH are shown to be as potent as natural GHRH. GHRH stimulates synthesis and secretion of growth hormone by somatotrophs. GHRH binds to specific cell-surface receptors that are coupled to adenylyl cyclase though G proteins. Cyclic AMP mediates the GHRH effect on GH gene transcription and regulation GH secretion. GHRH also has a mitogenic effect on somatotrophs (Brazeau et al., 1982; Guillemin et al., 1981; Mayo et al., 1983; Michel et al., 1983; Reul et al., 1990). Interestingly, a study has reported that the use of GHRH plasmid-based therapy in companion dogs diagnosed with spontaneously occurring malignancies like hemangiosarcoma and carcinoma survived 84% longer than the placebo treatment group. The GHRH/GH/insulin like growth factor (IGF-I) axis has been implicated in the overall improvement in quality of life of cancer patients (Colao et al., 1999) and in this study, intramuscular treatment with GHRH-plasmid followed by electroporation in dogs with various kinds of tumors improved cachexia-associated anemia and the quality of life. Thus, GHRH-plasmid appears to have a beneficial effect on survivability of dogs with spontaneous tumors and deserves further investigation (Bodles-Brakhop et al., 2008).

Ghrelin

Ghrelin is a 28-amino-acid peptide with *O*-n-octanoylation at the serine in the third position, a modification essential for biological activity. This post-translational modification is unique among known neuropeptides. Ghrelin is produced by the stomach in anticipation of a meal, but has stimulatory effects on GHRH neurons and GH secretion by the pituitary (Kojima et al., 1999; Cone et al., 2003). Ghrelin mRNA and peptide have been detected in human pituitary, indicating that ghrelin is also synthesized within the pituitary, where it may influence the release of GH in an autocrine and paracrine manner (Kamegai et al., 2001). However, the GH-releasing capacity of ghrelin was shown to decline with aging in dogs (Bhatti et al., 2006).

Somatostatin: Growth Hormone Release Inhibiting Hormone

Somatostatin-producing neurons within the CNS are concentrated mainly in the anterior periventricular region of the hypothalamus with nerve terminals in external lamina of the median eminence. However, somatostatin-producing neurons are distributed also in other regions of the CNS and outside the CNS in epithelial cells (D cells) of the gastric mucosa, small intestine, kidney, islets of Langerhans of the pancreas, and parafollicular cells of thyroid. Somatostatin is synthesized as a precursor of 116 amino acids that is processed proteolytically to peptides with 14 or 28 amino acids. Somatostatin exerts its effects by binding to specific receptors on surface of target cells, which interact with G proteins. G proteins mediate a number of intracellular events, namely inhibition of adenyl cyclase activity leading to suppression of cAMP accumulation and inhibition of calcium fluxes leading to enhanced K^+ conductance and hyperpolarization. The fall in intracellular calcium reduces hormone secretion. GHRH also exerts a direct antiproliferative effect, inhibiting DNA synthesis and cell replication by blocking epidermal growth factor-induced centrosomal separation. This action might occur by interfering with movement of microfilaments and by preventing microtubule disassembly by inhibiting calcium influx. Table 26.9 summarizes the wide biological effects of somatostatin (Brazeau et al., 1973; Lamberts, 1986; Montminy et al., 1984; Reichlin, 1983; Schmeitzel and Lothrop, 1990).

Octreotide

Somatostatin has a limited clinical utility because of its short half-life (2–3 minutes) and its multiple effects. Since exogenous somatostatin is rapidly cleared from circulation, a series of longer-acting somatostatin analogs have been developed. The somatostatin analogue octreotide has been used to help manage pituitary disorders (Klonoff and Karam, 1992). Octreotide (Sandostatin®; Novartis) has been employed in the management of acromegaly, gastrinoma, pancreatic beta

Table 26.9 Actions of somatostatin. See text for explanation of abbreviations.

Inhibits hormone secretion by:
Pituitary gland
  TSH, GH, PRL, ACTH
Gastrointestinal tract
  Gastrin
  Secretin
  GIP
  Motilin
  Enteroglucagon
  VIP
Pancreas
  Insulin
  Glucagon
  Somatostatin
Genitourinary tract
  Renin
Inhibits other gastrointestinal actions:
Gastric acid secretion
Gastric emptying
Pancreatic enzyme and bicarbonate secretion
Intestinal absorption
Gastrointestinal blood flow
AVP stimulated water transport
Bile flow

cell tumors, and glucagonoma in the dog. Another long-acting somatostatin analogue, lanreotide (Somatuline® Depot), is currently being researched for use in dogs and has been approved by FDA for treatment of acromegaly in humans.

Pegvisomant

Pegvisomant is a GH receptor antagonist used to treat acromegaly. Pegvisomant is a polyethylene glycol derivative of a synthetic GH and has reduced immunogenicity and prolonged plasma residence time. It does not cross the human blood–brain barrier, there by sparing inhibition of central nervous system GH actions (Veldhuis et al., 2010). Thus, pegvisomant influences GH secretion predominantly by its actions outside the blood–brain barrier, such as the suppression of IGF-I concentrations in peripheral tissues like liver.

Somatotropin: Growth Hormone

Structure and Secretion

Somatotropin (GH) is a polypeptide hormone with a molecular weight of approximately 22 kD. The principal form of human somatotropin is a single chain, 191-amino-acid hormone, as was published in 1969 by Li (Li, 1969). There is considerable species specificity for somatotropin (Kostyo and Reagan, 1976; Wallis, 1975), and human, bovine, and porcine somatotropin used clinically are now commercially produced by recombinant techniques. The sequence of canine growth hormone was first identified in 1994 and that of the cat was identified in 1995 (Ascacio-Martinez and Barrera-Saldana, 1994; Castro-Peralta and Barrera-Saldana, 1995).

Somatotropin is synthesized and released under the influence of growth hormone releasing hormone (GHRH), a 44-amino-acid peptide. The secretion of somatotropin occurs in a pulsatile fashion, which results from the asynchronous release of GHRH and somatostatin (growth hormone inhibition hormone, GHIH), a potent inhibitor of somatotropin release (Müller, 1987). Pulsatile release of GH appears to be the effect of GHRH pulsatile secretion, whereas between-pulse concentrations are largely determined by somatostatin (somatotropin-release inhibiting hormone, SRIH) (Rijnberk, 1996). Many physiological, pharmacological, and pathological factors influence the secretion of somatotropin, including exercise and hypoglycemia, which increase somatotropin secretion, as do α-adrenergic agonists, β-adrenergic antagonists, and dopamine agonists. Glucocorticoids, α-adrenoceptor blockers, and β-adrenergic agonists decrease somatotropin secretion, as do hyperglycemia and obesity. See Table 26.10 for a review of regulatory factors for growth hormone release (Cone et al., 2003).

Table 26.10 Factors influencing growth hormone secretion

Stimulate	Inhibit
Hypoglycemia	Hyperglycemia
Exercise	Free fatty acids
Sleep	Somatostatin
Amino acids	Growth hormone (negative feedback)
Hypoglycemia	Antidiuretic hormone (ADH)
Growth hormone–releasing hormone (GHRH)	
Ghrelin	
Adrenocorticotropic hormone (ACTH)	
α_2-adrenergic agonists (clonidine)	α-adrenergic antagonists
M1 cholinergic agonists	M1 cholinergic antagonists
β-adrenergic antagonists	β_2-adrenergic agonists
Dopamine or agonists	Dopamine antagonists (phenothiazines)
γ-aminobutyric acid (GABA) or agonists	
Histamine 1 agonists	H_1-histamine antagonists
Serotonin agonists	Serotonin antagonists
μ opioids	Neuropeptide Y (NPY)
Testosterone	Progesterone
Estrogen	
Stress glucocorticoids (acute)	Glucocorticoids (chronic)

Function

The receptor for GH is a cell surface receptor and belongs to the JAK/STAT cytokine receptor superfamily. GH has two distinct receptor binding sites and thus dimerization of GH receptors is required for the binding of GH and subsequent activation of the signaling cascade. Growth hormone's effects can be divided into two main categories: rapid or metabolic actions, and slow or hypertrophic actions (Rijnberk, 1996). Acute catabolic effects are mediated by GH by decreasing carbohydrate utilization and impairing glucose uptake into cells, which results in glucose intolerance and secondary hyperinsulinism (Lean et al., 1992). The slower anabolic effects are mediated via IGFs. These hormones are produced in many tissues, particularly liver, in response to somatotropin. Growth hormone, via IGF-1, increases protein synthesis, and decreases protein catabolism by mobilizing fat and increases lean body mass and bone density. This protein-sparing effect is important for linear growth and development. Furthermore, IGF-1 can elicit feedback inhibition to regulate both GHRH and GH secretion.

Therapeutic use

Small animal applications: In young dogs, growth hormone deficiency can occur as a primary endocrine abnormality resulting in pituitary dwarfism. It may be treated with bovine, porcine, or human somatotropin. Porcine somatotropin (pGH) is structurally identical to canine somatotropin and may be preferred for treatment in the dog (Ascacio-Martinez and Barrera-Saldana, 1994). The pharmacological and toxicological effects of exogenous porcine somatotropin administration in normal adult dogs was evaluated. The results indicated that pGH caused: a dose-dependent increase in body weight and the weights of liver, kidney, thyroid, and pituitary gland; increased skin thickness; and increased serum IGF-1 levels. However, minimal or no biologically significant effect of pGH on serum T_3, T_4, and cortisol levels in dogs was noticed (Prahalada et al., 1998).

Preparations and dosage protocols: It has been recommended to administer somatotropin at 0.1 IU/kg to dogs subcutaneously three times weekly for 4–6 weeks (Eigenmann, 1982). If ACTH and TSH secretion are also abnormal, the animals need to be treated with replacement doses of glucocorticoids and thyroid hormone. Growth hormone–responsive dermatosis has been treated successfully with 0.15 IU/kg two times weekly for 6 weeks (Schmeitzel and Lothrop, 1990).

Large animal applications: Bovine somatotropin (bST) has been used in cattle for several reasons. Somatotropin improves feed efficiency and leads to an effective increase in protein and reduction of fat, thus providing a desirable carcass in terms of degree of fatness and meat quality (Groenwegen et al., 1990; Schwarz et al., 1993). This has also been seen when bST was administered in finishing lambs (McLaughlin et al., 1993). In the pig, porcine somatotropin also leads to increased growth rates and improved meat quality (Campbell et al., 1988; Etherton et al., 1987). A study has indicated that use of bST was effective in reducing carcass fat and increasing edible lean of Holstein steers, but administering bST to young lightweight steers decreased carcass quality (Schlegel et al., 2006).

Treatment with bST was shown to improve fertilization rate, accelerate embryo development, and improve embryo survival. Presence of a conceptus altered endometrial expression of genes and proteins in response to bST to improve pregnancy rates (Moreira et al., 2002).

In addition to improvements in meat quality and fertility, bST has been used to increase milk production in the cow, and in the USA, this application has been approved since 1994. Somatotropin also has an effect on milk composition, resulting in less short- and medium-chain fatty acids and more long-chain fatty acids (Bauman, 1992, 1999; Lean et al., 1992).

Bovine milk naturally contains less than 1 ng/ml of GH whereas humans secrete 500–875 µg GH per day. GH does not significantly increase in milk when cows are treated with recombinant bST. Bovine growth hormone itself is degraded by the gastric acid when ingested orally and it is reported that the amino acid sequence of bST differs from that of hST by approximately 35% and as a result human growth hormone receptors do not respond to bST (bovine GH) (Lesniak et al., 1977).

Administration of bST increases IGF-1 concentrations in bovine milk, and some researchers have postulated that dairy products may increase the risk of breast cancer due to their content of fat, IGF-1, estrogens, or growth hormone. However, a metaanalysis review concluded that the available evidence does not support this association. Although IGF-1 concentrations are not affected by pasteurization, the average milk content of IGF-1 has been estimated at 4 ng/ml. Based upon a 1.5 l/day milk intake, this would contribute 6 µg of IGF-1 to the gastrointestinal tract. About 380 µg are contributed by gastrointestinal secretions. These amounts compare to the 10,000 µg produced daily in the liver and extrahepatic tissues. Thus, milk-derived IGF-1 is estimated to contribute less than 0.06% of the total IGF-1, and that would likely be an overestimate as it assumes that all IGF-1 survives proteolysis in the gastrointestinal tract (Parodi, 2005).

It is important to note, however, that the European Union (EU) currently has a moratorium banning the use of bST in order to increase milk yield. An immunoassay for IGF-1 concentrations in milk has been developed and proposed as a regulatory test in milk samples.

Preparations and dosage protocols: Several formulations of bovine somatotropin (bST) are on the market. These include a daily dose, and 7, 14, and 28-day prolonged release preparations. The 14 and 28-day preparations are methionyl bST preparations. Exogenous bST must be present every day in order to continue an augmented milk response. The reason is that the bST peptide is rapidly cleared from the body. When given to cattle daily, maximum milk response is achieved with bST doses of 30–40 mg/day, and no further increase is observed with higher doses. Most production trials have employed doses between 10 and 50 mg/day. Posilac® (Monsanto) is 500 mg of met^{-1} . . . leu^{126} recombinant bovine somatotropin (Sometribove, USAN), a 14-day prolonged release preparation of bST (Sometribove, USAN), which is administered every 14 days. Milk yield generally increases the first few days of bST treatment, reaching the maximum on about the sixth day. In swine, prolonged release (4–6 weeks) injectables or ear tags are also available (Bauman, 1992, 1999; Lean et al., 1992).

Adverse effects

In dogs, diabetes mellitus is the major potential side effect of growth hormone administration; therefore, fasting blood glucose concentrations should be determined before and at weekly intervals while on growth hormone therapy. Hypersensitivity reactions might also occur after treatment with growth hormone (Schmeitzel and Lothrop, 1990).

In cattle, treatment with growth hormone has been reported to cause an initial decrease in food intake; however, after a few weeks an increase is seen. It has also been suggested that growth hormone treatment increases the incidence of mastitis, which may be a function of the increase in milk production (Bauman, 1992, 1999; Lean et al., 1992). However, in a multinational study involving over 900 cows no effect on incidence of clinical mastitis was seen (White et al., 1994). There are no indications that growth hormone administration leads to an increase in metabolic diseases in this species (Bauman, 1992, 1999; Lean et al., 1992).

Mecasermin

Mecasermin (Increlex®; Cambrex Bio Sciences Baltimore, Inc.) is a recombinant human IGF-1. It is a single-chain, nonglycosylated 70-amino-acid protein connected via three disulphide bonds. The amino acid sequence of mecasermin is identical to the native IGF-1. Mecasermin is approved by FDA for treatment of severe IGF-1 deficiency that is not responsive to GH in humans. Use of mecasermin in veterinary medicine is still under research.

Prolactin

Structure and biosynthesis

Prolactin (PRL) is a 198-amino-acid polypeptide hormone with a molecular weight of 22,000, which is synthesized and secreted by the lactotrophs of the anterior pituitary. It is classified as a somatomammotropic hormone and about 10–15% of the molecule represents glycosylation. Although glycosylated PRL is less potent, the glycosylation appears to stabilize the molecule to degradation in the body. In the species for which its structure has been identified, PRL has three intrachain disulfide bridges. The amino acid sequences of canine and feline prolactin have been identified (Gomez-Ochoa et al., 2004; Warren et al., 1996).

There are no pharmaceutical preparations of this hormone and few, if any, therapeutic applications. Prolactin regulation is of relevance to the reproductively cycling animal in a variety of species. PRL evolved from a hormone common to GH and human placental lactogen (hPL), but it now shares only a minority of residues (13 and 16%, respectively) with these hormones. The precursor molecule for PRL is a 227 amino acid with a molecular weight of 40,000 to 50,000 and contributes to some of the plasma PRL immunoreactivity (Klonoff and Karam, 1992; Tyrell et al., 1994).

Function

PRL stimulates lactation in the postpartum period. It appears to have functions related to reproduction, particularly related to the care, feeding, and protection of offspring, even in fish and birds. Prolactin seems to have effects on salt and water metabolism, and is important in the ability of the salmon, for example, in changing from a saltwater to a freshwater environment. In mammals, the increasing concentrations of prolactin during pregnancy, combined with the hormonal effects of estrogens and progestins, induce the development of mammary tissue. In rodents, prolactin prolongs the life of the corpus luteum, but does not perform this function in humans or domestic mammals. The secretion of prolactin with suckling in certain species, most notably humans, is also likely to inhibit ovarian function and prevent ovulation and fertility (Klonoff and Karam, 1992; Tyrell et al., 1994).

Regulation of secretion

Secretion of prolactin by the pituitary is normally under inhibitory control by the hypothalamus, specifically by the neurotransmitter dopamine. Therefore, dopamine agonists inhibit prolactin secretion very effectively in hyperprolatinemia. Dopamine agonists like bromocriptine (Cycloset®) shrink pituitary prolactin-secreting tumors, lower circulating prolactin levels and restored ovulation in 70% of women with microadenomas (Webster et al., 1994), and suppress PRL and hence progesterone in pregnant bitches (Onclin et al., 1995). Factors influencing secretion of growth hormone often have similar effects on PRL: sleep, stress, hypoglycemia, and exercise all increase PRL and GH. Other PRL-releasing hormones include TRH, which may explain galactorrhea in some hypothyroid bitches. Also the dopamine antagonists phenothiazines and metoclopramide, as well as butyrophenones, increase prolactin secretion. The half-life of prolactin in plasma is only 15–20 minutes. A prolactin inhibiting hormone (PRIH) of peptide nature has been investigated in rats. In humans, heavy athletic training, likely through opioid release, may inhibit PRL production and result in reproductive alterations, specifically amenorrhea in women (Kuret and Murad, 1990).

Therapeutic uses

There are no applications of clinical relevance. Milk production in cattle does not seem to be limited by the usual concentrations of circulating prolactin. However, research has focused upon the ability of GH to be galactopoietic.

Posterior Pituitary Hormones

There are two known posterior pituitary hormones: vasopressin (antidiuretic hormone, ADH) and oxytocin. Both hormones differ from vasotocin found in nonmammalian vertebrates by only one amino acid. Posterior pituitary hormones are synthesized in the hypothalamus and transported to the posterior pituitary where they are stored and released into the circulation.

Antidiuretic Hormone

Two nine-amino-acid peptides are secreted by the neurohypophyseal system: ADH (or arginine vasopressin, AVP) and oxytocin. They are synthesized by the magnocellular neurons of the supraoptic nuclei and the lateral and superior parts of the paraventricular nuclei. ADH regulates the water permeability of the distal tubules and collecting duct of the nephron. It also is a vasoconstrictor and influences cardiovascular function.

Structure

Antidiuretic hormone has a molecular weight of 1084 and is characterized by a six-amino-acid ring and a three-amino-acid side chain with a disulfide linkage. The prohormone for ADH includes the ADH sequence and neurophysin II, which binds to ADH. Following synthesis of the peptide, secretory granules containing the prohormone move down the axon to the nerve terminal in the posterior lobe of the pituitary. Upon exocytosis, equimolar amounts of the neurophysin and ADH are released. The neurons also project to the choroid plexus where they also release ADH into the cerebrospinal fluid.

Stimuli for Release

The primary physiological stimulus for ADH release is an increase in plasma osmolality. However, hypovolemia, pain, exercise, and some drugs may stimulate ADH release.

Mechanism of Action

Three receptor subtypes that mediate the actions of AVP have been identified (V_{1A}, $V_{1B,}$ and V_2). V_{1A} receptors are located in vascular smooth muscle cells and the heart, and mediate vasopressin-induced vasoconstriction and increased afterload. The V_{1B} receptor is located in the anterior pituitary and mediates ACTH release. V_2 receptors are found on the renal tubule cells and are involved in the mediation of antidiuresis through increased water permeability and water reabsorption in the collecting ducts. ADH increases cyclic AMP in the tubule, which increases water permeability by stimulating the insertion of aquaporin 2 proteins at the luminal surface, resulting in increased water permeability and reabsorption, urine osmolality, and decreased urine volume. V_2 receptors outside of the kidney also mediate the release of the coagulation factor VIII and von Willebrand's factor. Aside from its value in treating conditions like central diabetes insipidus where vasopressin is deficient, irreversible shock has also been postulated to be exacerbated by a vasopressin deficiency, leading to its use in shock therapy.

Desmopressin acetate (DDAVP, 1-desamino-8-D-arginine vasopressin) is a long-acting synthetic analogue of predominately V_2 and is a potent antidiuretic. Several nonpeptide AVP antagonists, like vaptans, conivaptan (Vaprisol®), and tolvaptan (Samsca^{TM}), have been developed, and are being evaluated, and are approved by the FDA, for administration in hyponatremia and fluid overload in humans (Ali et al., 2007).

Table 26.11 summarizes the actions of vasopressin and the respective ADH receptors (V_{1A}, V_{1B}, and V_2) mediating the actions.

Absorption, Metabolism, and Excretion

Vasopressin must be administered parenterally and has a very short half-life of about 20 minutes.

Preparations

Natural and synthetic ADH is available commercially for the diagnosis and treatment of diabetes insipidus. Dogs, cats, horses, and humans produce arginine-ADH and pigs produce lysine ADH. Desmopressin or deamino-D-arginine vasopressin (DDAVP) is available for parenteral (subcutaneous) injection; the acetate form is available for nasal administration and as 0.1 and 0.2-mg tablets.

Table 26.11 Actions of vasopressin

Target organ	Receptor type	Action
Renal glomerulus	V_1	Mesangial cell contraction
Vasa recta	V_1	Decrease medullary blood flow
Juxtaglomerular cells	V_1	Suppress renin release
Arterioles	V_1	Constriction
Liver	V_1	Increase glycogenolysis
Anterior pituitary	V_1	Increase ACTH release
Baroreceptors	?	Desensitization or sensitization of baroreflex
Cortical and medullary collecting tubules	V_2	Increase H_2O permeability
Papillary collecting ducts	V_2	Increase H_2O permeability
Thick ascending loop of Henle	V_2	Increase Na^+, K^+, Cl^- reabsorption

Diagnosis of Diabetes Insipidus

Diabetes insipidus (DI) is caused by the deficiency of antidiuretic hormone (central DI) or by absence of a renal response to this anterior pituitary hormone (nephrogenic DI). The main presenting signs, in the absence of other conditions, are polyuria and polydipsia.

Central DI is due to the absolute deficiency of antidiuretic hormone (vasopressin; ADH). In partial central DI, some endogenous ADH secretion remains. These conditions are the result of the destruction of the supraoptic and paraventricular nuclei of the hypothalamus, which have axons terminating in the posterior pituitary. The damage may be due to head trauma from surgical transection of the pituitary stalk (usually only transient DI) or primary or metastatic tumors, or (most commonly in veterinary medicine) the cause is not known (idiopathic DI).

Nephrogenic diabetes insipidus results when the renal tubule is insensitive to ADH. In this condition, ADH does not increase intratubular cAMP concentrations, a necessary prerequisite to the increased water permeability normally induced by ADH. This abnormality of ADH responsiveness may also be partial or total. Primary causes of nephrogenic diabetes are rare. However, secondary nephrogenic DI may result from pyometra, liver disease, hyperadrenocorticism, hyperthyroidism, hypercalcemic disorders, renal failure, and pyelonephritis.

Diabetes insipidus is diagnosed if the urine specific gravity is dilute (less than 1.008) in the face of dehydration and/or an elevated plasma osmolality. However, it is not infrequent for the animal to present in a hydrated state and to have a normal or only slightly elevated plasma osmolality. A modified water deprivation test is recommended to confirm that endogenous ADH and urine osmolality will not rise in the face of moderate dehydration. Following carefully monitored gradual water withdrawal over 3 days, water is completely withdrawn on the 4th day and the urine osmolality and plasma osmolality are monitored. If greater than 5% dehydration is achieved and no urine concentration is observed, exogenous ADH is administered to test the ability to respond to exogenous hormone.

Vasopressin can be administered in the following manner in this test:

The administration of 1 milliunit/ml of aqueous ADH (Vasostrict®, Par Sterile Products Vasopressin USP) in Lactated Ringer's or 5% dextrose. This solution is administered IV over 1 hour at the rate of 10 ml per kg body weight. Urine samples should be obtained at 15-minute intervals for 90 minutes following ADH administration.

In an animal with complete central DI, the urine osmolality will not have risen above isoosmolality (300 mOsm per kg) with dehydration, and subsequent ADH administration will increase urine osmolality at least 50%.

In an animal with partial central DI, the urine osmolality will increase above isoosmolality, but it will increase an additional 10–50% following exogenous ADH.

Animals with nephrogenic DI do not concentrate their urine upon dehydration above isoosmolality, and also do not respond to exogenous ADH.

Therapeutic Uses

Aqueous ADH or ADH analogues are currently the only formulations available for the treatment of total and partial central DI. Synthetic ADH analogues DDAVP (DDAVP®, Stimate®, Minirin®, Ferring Pharmaceuticals) and LVP (lysine-8-vasopressin, Lypressin®, Diapid Nasal Spray®, Novartis) are the most commonly used. Both of these preparations can be administered intranasally or into the conjunctival sac. The latter route appears to be better tolerated by the animals. Ocular or conjunctival irritation is a rare problem.

DDAVP is a drug with greater potency and slower metabolism than the natural ADH molecule. Administration of 5–20 μg of DDAVP (2–4 drops) in single or divided doses controls polyuria in most animals. The peak drug action is seen at 2–6 hours and its duration may last from 10 to 27 hours. The clear advantage of this medication is that it does not require parenteral administration. However, the conjunctival route results in variable amounts of drug reaching the blood stream and variable duration of effect even in the same patient. DDAVP is also quite expensive and therefore it might be prudent to use the drug only when polyuria is observed or to avoid excessive nocturnal urine production.

LVP is another product that is available for managing DI via nasal or conjunctival administration. However, its duration of action is shorter and expense greater than the other products. As a result, it has not found much application in veterinary medicine (Chastain and Ganjam, 1986; Ferguson et al., 1992).

DDAVP has also been used for bleeding disorders (von Willebrand's disease and hemophilia A). In pharmacological doses, it increases plasma levels of factor VIII:C and von Willebrand factor, via preferentially increasing levels of larger von Willebrand factor multimers and by increasing platelet adhesion. Controlled studies on the use of DDAVP in dogs are lacking, but the clinical impression is that DDAVP is beneficial in some, but not all, von Willebrand's dogs. The initial parenteral dose of DDAVP (with either preparation) is 0.5–2 μg SQ once or twice daily. In animals, administration as eye drops is often more convenient. For chronic therapy of central diabetes insipidus, the DDAVP tablets are dosed initially at 0.1 mg given three times a day. The dose is gradually increased to effect if there still is unacceptable polyuria and polydipsia persisting a week after starting therapy. Most dogs require 0.1–0.2 mg of DDAVP two to three times a day for chronic therapy.

Toxicity

Immediately following a dose, in order to avoid water intoxication, dogs should not be given unlimited quantities of water. The transiently high levels of ADH will prevent the excretion of a free water load by the kidney and result in overhydration and possible neurological sequela such as cerebral edema. Cerebral edema may be manifested by depression, vomiting, salivation, ataxia, muscle tremors, and convulsions. Animals with central or nephrogenic DI disease may also be successfully managed by providing free access to water at all times and by housing the animals outdoors. Another inexpensive maneuver that reduces the urine output is the restriction of dietary sodium using homemade diets or the commercial diets designed for use in congestive heart failure (e.g., Hill's H/D). Such products generally contain less than 0.1% sodium on a dry weight basis (Ferguson et al., 1992).

Other Drugs for Treatment of Central Diabetes Insipidus

Oral agents have also been used primarily as adjuncts to ADH therapy of central DI. Chlorpropamide (Diabinese®, Pfizer), a sulfonylurea hypoglycemic agent used to treat noninsulin-dependent diabetes in humans, has produced inconsistent antidiuretic effects in the dog and cat. Chlorpropamide's effect is to enhance the effect of ADH on the renal tubules and collecting duct by increasing intracellular cyclic AMP. It may also stimulate pituitary ADH release. As a result, it is only effective in the presence of sufficient endogenous (partial central DI) or exogenously administered ADH. Careful dosage studies for chlorpropamide have not been performed in the dog. Reported doses include 250 mg every 12 hours and 10–40 mg/kg/day. Reduction in urinary volumes ranging from 18 to 50% has been reported. Maximal antidiuretic effects take 1–2 weeks to develop. Side effects of hypoglycemia can be minimized by frequent feedings and periodic monitoring of blood glucose concentrations.

Carbamazepine (Tegretol®, Novartis), an antiepileptic, is also effective in some cases of central DI. In contrast to the other drugs, these agents may increase the secretion of ADH and therefore would be rational therapy only in partial central DI. Likewise, indapamide (Mylan Pharms Inc.), an antihypertensive diuretic drug that increases urinary osmolality and decrease serum osmolality, appears to be effective in the mild form of central DI. However, there have been no reports in the veterinary literature of the use of these drugs for the successful treatment of DI.

Thiazide diuretics when used together with salt restriction may serve to potentiate the effect of exogenous or endogenous ADH (see Section Treatment of Nephrogenic Diabetes Insipidus) (Ferguson et al., 1992; Klonoff and Karam, 1992; Tyrell et al., 1994).

Treatment of Nephrogenic Diabetes Insipidus

Treatment for nephrogenic DI should, if possible, start with correction of the underlying cause of the nephrogenic DI (hypercalcemia, renal infection, hyperadrenocorticism without a compressive pituitary tumor). Except for institution of a low-sodium diet, the thiazide diuretics are the only agents that have been shown to be effective in the treatment of nephrogenic DI.

Thiazide diuretics have a paradoxical antidiuretic effect in central and nephrogenic DI. These agents may reduce the reabsorption of sodium in the ascending loop of Henle, resulting in enhanced urinary sodium loss, mild reduction in plasma osmolality, and, therefore, diminished thirst. The reduction in water intake causes contraction of the extracellular volume, and proximal tubular sodium reabsorption is increased and the glomerular filtration rate decreased. The urine volume is thereby reduced without overt concentration of the urine osmolality. Hydrochlorothiazide (Microzide®, Watson Labs) at a dosage of 2.5–5 mg/kg has succeeded in reducing water intake by 50–85% in cases of ADH-resistant polyuria. Due to the kaliuretic effect of the thiazides, serum potassium should be monitored and oral potassium gluconate administered if the animal becomes anorexic (Ferguson et al., 1992). However, in human patients, combined use of thiazide diuretics and selective serotonin reuptake inhibitors synergistically impaired renal free water clearance resulting in severe hyponatremia (Rosner, 2004).

Oxytocin

Oxytocin, like vasopressin, is a nine-amino-acid peptide with an intrapeptide disulfide cross-link. It induces contraction of smooth muscle, most importantly of the myoepithelial cells of the mammary gland, which results in milk ejection. Furthermore, it also results in uterine smooth muscle contraction, an effect that increases during pregnancy. Oxytocin increases the release of prostaglandins and leukotrienes that augments the uterine smooth muscle contraction. Its clinical use in the management of milk letdown and uterine contraction (induction of parturition and treatment of pyometra) is described in detail in Chapter 27. Oxytocin is also involved in endocrine and neuroendocrine regulation through receptor-mediated actions exerted on the heart, vasculature, and kidneys (Gutkowska et al., 1997; Jankowski et al., 2000; Conrad et al., 1993).

Mechanism of Action

Oxytocin acts through G-protein coupled receptors and affects the transmembrane ionic currents in uterine smooth muscle cells resulting in sustained uterine contraction. Oxytocin-induced myometrial contractions can be inhibited by tocolytic agents such as β-adrenergic agonists, magnesium sulfate, and inhalation anesthetics (Klonoff and Karam, 1992; Tyrell et al., 1994). Oxytocin receptor antagonist atosiban (Tractocile®) is indicated for tocolysis in preterm labor outside the United States; however, this drug is not tested for veterinary use.

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27

Hormones Affecting Reproduction

Wolfgang Bäumer, C. Scott Bailey, and John Gadsby

Introduction

The brain (hypothalamus) regulates overall reproduction in animals via the synthesis and release of several peptide hormones. It is affected by the physiological status of the animal, and (depending on species) by environmental cues. The best-described and most potent environmental factor is day length, which affects gonadotropin secretion by inhibiting melatonin secretion. Hypothalamic hormones are released from hypothalamic neurons in the region of the median eminence, and they reach the anterior pituitary through the hypothalamic–adenohypophyseal portal system. The two main hypothalamic hormones relevant to reproduction are gonadotropin releasing hormone (GnRH) and the prolactin-inhibitory hormone known as dopamine. The primary function of these hormones is to stimulate or inhibit the release of specific anterior pituitary hormones.

The pituitary gland is essential for the regulation of reproduction, as well as growth, stress, and intermediary metabolism. The pituitary gland consists of three separate lobes in vertebrates: the *anterior (adenohypophysis), posterior (neurohypophysis),* and *intermediate lobes.* The anterior pituitary lobe releases two distinct types of reproductive "trophic" hormones: the gonadotropins (follicle stimulating hormone, FSH, and luteinizing hormone, LH) and prolactin. Based on evolutionary and structural considerations, FSH and LH are grouped together and are glycoprotein hormones, while prolactin is related to growth hormone and is known as a somatomammotropin. A large number of reproductive states as well as a diverse group of drugs also affect their secretion and function (Wright and Malmo, 1992; Driancourt, 2001; Wiltbank et al., 2011).

Steroid hormones play vital roles in reproduction. The ovary (females) and testis (males) are the primary sources of reproductive steroid hormones in animals. The gonadotropins, FSH and LH, which are secreted in response to hypothalamic GnRH, stimulate the secretion of gonadal steroids (primarily estrogens and progesterone in the female, and testosterone and estrogen in the male). A list of hormones and drugs affecting reproduction is summarized in Table 27.1.

Estrous Cycle

In females, GnRH from the hypothalamus and the gonadotropins, FSH and LH, from the pituitary, regulate the estrous cycle (Figure 27.1). GnRH secreted from the hypothalamus is transported by the hypophyseal portal blood vessels to the pituitary gland, where it increases secretion of the gonadotropins, FSH and LH. Gonadotropins stimulate the secretion of gonadal steroids (estrogens and progesterone). Estrogens and progesterone have prominent stimulatory effects upon the female reproductive tract and the mammary gland, while increased circulating levels of these steroids decrease gonadotropin secretion (via negative feedback). Each estrous cycle consists of the following phases:

1) Follicular phase (= proestrus and estrus): during the follicular phase ovarian follicles (one or more depending on the species) develop and mature, secreting increasing amounts of estrogen. These increased amounts of estrogen trigger a surge in release of LH from the pituitary gland, causing ovulation of follicles and the release of ova into the oviduct (see ** in Figure 27.1; except in the queen and in camelids, which are induced ovulators).
2) Luteal phase (= metestrus and diestrus): following ovulation the remnants of each follicle develop into a corpus luteum, whose primary function is secretion of progesterone, which is required to act on the uterus to create an environment compatible with attachment/ implantation of the embryo. If the ova are not fertilized, or if embryos fail to develop or attach/ implant, the corpus luteum regresses (degenerates) in

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Table 27.1 Reproductive hormones and related agents

Hormone	Indications
Gonadotropin releasing hormone (GnRH) and gonadotropins	
GnRH	Ovulation induction, infertility therapy
Gonadorelin (synthetic GnRH)	Ovulation induction, infertility therapy
Follicle stimulating hormone (FSH)	Follicle development for embryo transfer
Human chorionic gonadotropin (hCG)	Ovulation induction, infertility therapy
Equine chorionic gonadotropin (eCG)	Ovulation induction, infertility therapy
Oxytocics (ecbolics = uterotonics)	
Oxytocin	Labor induction, milk letdown
Progestins	
Altrenogest	Synchronization of estrus in mare and pig
Melengestrol acetate (MGA)	Synchronization of estrus in cattle
Progesterone (injectable or intravaginal delivery – CIDR)	Synchronization of estrus in cattle, sheep, goats, and mare
Androgens	
Nandrolone	Catabolic disease states in horses and dogs
Stanazolol	Catabolic disease states in horses and dogs
Antiandrogens	
Finasteride	Benign prostatic hypertrophy in dogs
Prostaglandins	
Lutalyse®	Regulation of the estrous cycle in ruminants (e.g., cows) Induction of abortion (various species)
Estrumate® (cloprostenol)	Induction of parturition in sows Induction of abortion (various species)

most species, leading to the cessation of progesterone secretion. However, during pregnancy, the life-span of the corpus luteum is lengthened (except in the bitch), resulting in continued progesterone secretion, which is necessary for pregnancy maintenance.

In nonpregnant polyestrous animals, during the late stages of the luteal phase of the estrous cycle, the uterus (endometrium) releases a hormone called prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). This hormone causes corpus luteum regression and a decline in progesterone secretion. The withdrawal of progesterone removes negative feedback control on the hypothalamus/ pituitary, allowing increased gonadotropin secretion and the initiation of another estrous cycle (follicular phase followed by luteal phase). It is not secreted in detectable amounts in nonpregnant domestic carnivores (dogs and cats), resulting in a much longer return to estrus in these species. During the breeding season, estrus (female reproductive behavior induced by follicular estrogen) follows corpus luteum regression within a few days.

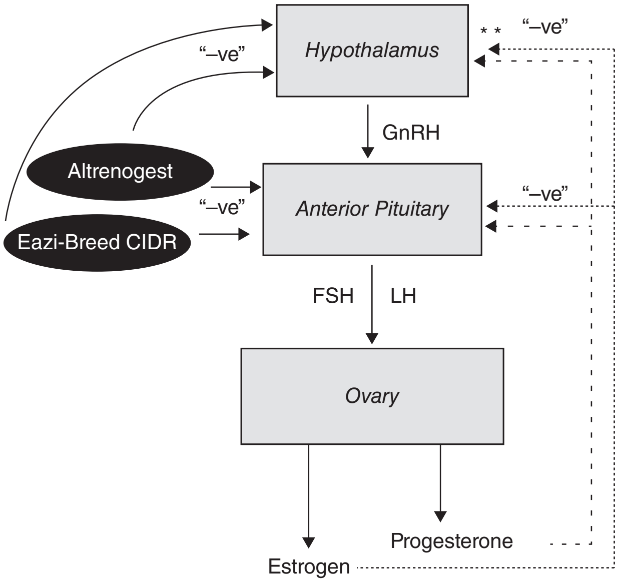

Figure 27.1 Regulation of estrous cycle and gonadal hormone secretion. Gonadotropin-releasing hormone (GnRH) stimulates gonadotropin (follicle stimulating hormone, FSH; luteinizing hormone, LH) release, which subsequently increases ovarian steroid hormone production during the estrous cycle. Like natural estrogen and progesterone, synthetic agents such as altrenogest and melengestrol acetate (MGA) control the estrous cycle by *negative feedback* mechanisms. Note: ** indicates that estrogen at high levels during the follicular phase of the natural estrous cycle, induce a positive feedback effect on the hypothalamus to generate GnRH and LH-surges, resulting in ovulation. CIDR, controlled internal drug release.

The onset and maintenance of cyclicity are coordinated by a complex set of negative and positive feedback mechanisms. Consequently, therapeutic administration of natural or synthetic hormones have been used extensively in the control and synchronization of estrous cycles for breeding purposes (discussed in the sections on each drug in this chapter). Progesterone-like hormones (progestins; e.g., MGA or altrenogest) are the frequently used hormones in theriogenology (Wright and Malmo, 1992).

Gonadotropin-Releasing Hormone, Gonadorelin, and Gonadotropins

The neurohormones such as GnRH (and analogs), gonadotropins (FSH and LH), prolactin and oxytocin, that are important in veterinary medicine are discussed here.

Gonadotropin-Releasing Hormone

Structure and Function

Gonadotropin-releasing hormone (GnRH) is the decapeptide hypothalamic-releasing hormone responsible for stimulating the release of gonadotropins, FSH and LH, by the anterior pituitary gonadotropes. GnRH has a very short half-life (2–4 minutes), is released intermittently (in pulses) and its release is controlled by a neural pulse generator in the hypothalamus. Such intermittent release is crucial for the proper synthesis and release of the gonadotropins, which also are released in a pulsatile fashion (Peters, 2005). Both gonadotropins and gonadal steroids regulate GnRH production in a negative feedback manner (Peters, 2005).

Mechanism

GnRH stimulates the synthesis and release of gonadotropins by binding to the GnRH receptor, a G protein-coupled receptor linked to the IP_3-Ca^{2+} signal transduction pathway. Pulsatile or episodic administration of GnRH stimulates the secretion of gonadotropins and forms the basis of infertility therapy and ovulation induction by increasing gonadal stimulation (see Section Clinical Uses, i). Alternatively, continuous administration of GnRH leads to desensitization and down-regulation of GnRH receptors on pituitary gonadotropes. This leads to the suppression of gonadotropin secretion and forms the basis for the clinical use of long-acting GnRH analogs (e.g., *gonadorelin*) to cause medical castration (see Section Clinical Use, ii) (Stout and Colenbrander, 2004).

Clinical Uses

The two principal uses of GnRH are: (i) induction of ovulation or follicular luteinization, and (ii) suppression of gonadotropin secretion (medical castration). A number of clinical GnRH analogs have been synthesized. These include synthetic GnRH (gonadorelin and buserelin) and other potent, long-acting GnRH analogs (e.g., deslorelin).

Gonadorelin

Gonadorelin (Cystorelin® or Factrel®) is a synthetic preparation of GnRH used to treat animals that fail to ovulate or develop follicular cysts. Deslorelin is available for horses and dogs as a SQ implant (Ovuplant®) or injectable formulation (Sucromate®). Both stimulate the synthesis and secretion of FSH and LH by interacting with GnRH receptors on the pituitary gonadotropes. However, the continuous exposure to gonadorelin or deslorelin leads to desensitization and down-regulation of GnRH receptors on pituitary gonadotropes, which may be the basis for its clinical use to limit estrus in some animals. Thus, the response to these GnRH analogs depends on the dose and time course (first stimulatory, then long-term inhibitory) (Thatcher et al., 2001; Johnson et al., 2002).

Uses

1) Ovulation induction. Gonadorelin has been used empirically to induce ovulation at the time of breeding in both cattle and horses, and is a component of the "Ovsynch" protocol. Due to physiological differences (a prolonged LH surge, lasting 24–36 hours) in horses, a single dose of gonadorelin is insufficient for reliable ovulation induction, and a long-acting formulation of deslorelin is more commonly used (Ferris et al., 2012). Gonadorelin has also been advocated for treatment of stallions with lowered libido. It is used in pulse-dosing to induce estrus in dogs and in cats with prolonged anestrus.
2) Cystic ovaries therapy. Gonadorelin is the drug of choice for treatment of *ovarian follicular cysts* ("cystic ovaries") in cattle (and camelids). Ovarian follicular cysts in cows are defined as follicle-like structures that persist rather than ovulate. These are more than 25 mm in diameter and have been present for 10 days or more in the absence of a corpus luteum. Their occurrence is frequent in the postpartum dairy cow, but rare in beef cows (Farin and Estill, 1993). The first choice for treatment is 100 μg GnRH, which generally results in luteinization of the cystic structure, with estrus occurring in 18–23 days. The administration of $PGF_{2\alpha}$ 9 days after GnRH will often shorten the interval to estrus (see Section Prostaglandin Analogs).
3) Estrus synchronization in conjunction with $PGF_{2\alpha}$: "Ovsynch" or "timed artificial insemination (AI)" protocol in cows (Lucy et al., 2004; Lamb et al., 2010; Wiltbank et al., 2011): Day 0, 100 μg GnRH; Day 7, 25 mg $PGF_{2\alpha;}$ Day 9, 100 μg GnRH; breed cattle by AI 20–24 hours after second GnRH. This allows synchronization of estrous cycles for AI without estrus detection.
4) Timed embryo transfer protocol, for recipient cows (Al-Katanani et al., 2002): using the "Ovsynch" treatment protocol as above, but instead of AI, transfer embryos into recipients on day 16–17. This enables preparation of recipients and embryo transfer without the need for estrus detection.
5) Gonadorelin is used in ferrets and induced ovulators (cats and camelids) to terminate estrus.
6) Gonadorelin can be used experimentally for diagnostic purposes to differentiate between pituitary and hypothalamic defects in dogs with hypogonadotropic hypogonadism.

7) Gonadorelin is also used to identify intact, from spayed or neutered, animals by estimating gonadorelin-stimulated release of FSH and LH.
8) Long-acting GnRH agonists (Ovuplant®) have been used successfully to inhibit cyclicity in domestic dogs and cats (Gobello et al., 2007).

Dosage

Veterinary products are listed in Table 27.2.

Gonadotropins

Secretion and Function

The pituitary hormones, FSH and LH, as well as the related hormones, *human chorionic gonadotropin* (hCG) and *equine chorionic gonadotropin* (eCG or pregnant mares serum gonadotopropin, PMSG), are referred to as the "gonadotropic" hormones. Each hormone is a glycosylated heterodimer containing a common α-subunit and a distinct β-subunit that confers specificity of action. A single hypothalamic releasing factor, GnRH, controls the synthesis and release of pituitary gonadotropins, LH and FSH, in males and females. LH and FSH are synthesized and secreted by gonadotropes, which make up ~20% of anterior pituitary cells. Gonadal steroid hormones (androgens, estrogens, and progesterone) cause feedback inhibition at the level of the pituitary and the hypothalamus to decrease pituitary gonadotropin secretion. The preovulatory surge of estrogen also can exert a stimulatory effect on the hypothalamus and thus promote pituitary gonadotropin surge release (Day, 2004). hCG is produced only in primates and is synthesized by syncytiotrophoblast cells of the placenta. eCG is produced only in equids and is secreted from the endometrial cups of pregnant mares in early pregnancy.

- *In males,* LH acts on testicular Leydig cells to stimulate the synthesis of androgens, primarily testosterone. FSH acts on the Sertoli cells to stimulate the production of proteins and nutrients required for the regulation of sperm production and maturation.
- *In females,* FSH and LH stimulate the growth and development of ovarian follicles, and thereby stimulate the follicle to produce estrogen, while LH induces ovulation and stimulates the developing corpus luteum (after ovulation) to secrete progesterone.

Mechanism

The actions of LH are mediated by the LH receptor, and those of FSH are mediated by the FSH receptor. Human chorionic gonadotropin and eCG variably stimulate one or both of the receptors, with the primary response being mediated via the LH receptor in most species. Interestingly, the chorionic gonadotropins do not reliably stimulate ovulation in the species of origin. Both of these G protein-coupled receptors are linked to adenylate cyclase and raise the intracellular levels of cAMP. There is a distinct species specificity for FSH and LH, which might lead to diminished efficacy or antibody generation in other species. hCG in particular has been shown to result in antibody production in horses (Roser et al., 1979) and has been associated with long-term or permanent infertility in some cats.

Therapeutic Uses

Apart from diagnostic application in pregnancy detection kits (i.e., hCG in early pregnancy tests for humans), gonadotropins are used in (i) promoting female and male fertility and (ii) treating cryptorchidism (Table 27.2).

The gonadotropin preparations that are available and commonly used clinically in animals are: human chorionic gonadotropin (hCG), equine chorionic gonadotropin (eCG), and follicle stimulating hormone (Follitropin-V) (Table 27.2).

Human chorionic gonadotropin

Human chorionic gonadotropin (hCG) is a gonadal stimulating hormone obtained from the urine of pregnant women. It is synthesized by syncytiotrophoblast cells of the placenta. It mainly possesses LH-like activity; therefore, it serves as a substitute for LH to promote follicle maturation, ovulation, and formation of corpus luteum. hCG is a glycoprotein and nonpituitary gonadotropin with long-lasting biological effects (>24 hours). A single injection is adequate for most reproductive uses. For example, because of its predominant LH-like activity, hCG is used to induce ovulation in the mare after an appropriate follicular size has been achieved (Wathes et al., 2003).

Uses

1) hCG is widely used for *infertility therapy*. It is used in female horses for hypogonadism due to pituitary hypofunction, and to *hasten or induce ovulation (*Samper, 2001).
2) hCG is used for the treatment of *cystic ovaries* in cows, by luteinizing the cystic structures to form a corpus luteum. The corpus luteum either regresses naturally or can be induced to regress with $PGF_{2\alpha}$ analogs (see Section Prostaglandin Analogs). Treatment recommendations are either hCG 5000 IU IV or 10,000 IU IM. Most cows respond with the establishment of an estrous cycle within 3–4 weeks.
3) eCG (400 IU) and hCG (200 IU) together make up PG600®, which is used in pigs to promote follicle development and induce estrous cycles in prepubertal gilts.
4) hCG is useful for treating male infertility due to *impotence*, particularly in the stallion. In the male,

Table 27.2 Drugs affecting reproduction in animals

Class	Preparation		Dosage
Gonadotropins			
Follicle stimulating hormone	Follitropin®-V		
	Ovagen®		
Equine chorionic gonadotropin (eCG)	PG600®	Combination of 400 IU eCG, 200 IU hCG	Pig: 1 ml PG600 IM
Human chorionic gonadotropin (hCG)	Follutein®	Injection 5,000 U and 10,000 U	Dog: 50–100 μg, SC, IV
	Chrorulon®		Cat: 25 μg, IM Horse:1,000 U, IV Cattle: 1,000–2,500 U, IV Sheep: 400–800 U, IV, IM Goat: 3,000 U, IV
Gonadorelin (synthetic GnRH)	Cystorelin®	Injection 50, 100 μg/ml	Dog: 50–100 μg, SC, IV
	Factrel®		Cat: 25 μg, IM Horse: 50 mg, SC Cattle: 100 mg, IM (100 μg)
Oxytocics			
Oxytocin	Pitocin®	Synthetic oxytocin injection 20 U/ml	Dog: 5–20 U, IM or IV once
	Syntocinon®		Cat: 2.5–5 U, IM once Pig: 10–20 U, IM Horse: 50–100 U, IV, IM, SC Cattle: 50–100 U, IV, IM, SC Sheep: 30–50 U, IV, IM, SC
Progestins			
Altrenogest	Regumate®	Solution: 2.2 mg/ml	Horse: 0.044 mg/kg/day for 15 days
Progesterone	Eazi-Breed CIDR™	Vaginal drug delivery devices	Cattle: 1.38 g for 7 days
Androgens			
Stanazolol	Vinstrol-V®	Tablets: 2 mg	Horse: 0.55 mg/kg, IM up to 4 doses once weekly
		Injection: 50 mg/ml	
Antiandrogens			
Finasteride	Proscar®	Tablets	Dog: 0.1–0.5 mg/kg, once daily for up to 16 weeks
Prostaglandins ($PGF_{2\alpha}$ agents)			
Dinoprost	Lutalyse®	Vials: 5 mg/ml	Cattle: 25 mg, IM injection Pig: 10 mg, IM injection Horse: 1 mg/100 lb body weight, IM injection Dog: 0.1–0.2 mg/kg daily for 5 days SQ (pyometra) 0.025–0.05 mg/kg q 12 h IM (termination of pregnancy) Cats: 0.1–0.25 mg/kg daily for 5 days SQ (pyometra) 0.5–1 mg/kg IM for 2 injection (termination of pregnancy)
Cloprostenol	Estrumate®	Vials: 0.25 mg/ml	Cattle, horse: 0.5 mg, IM injection

hCG stimulates the interstitial cells to produce testosterone.

5) hCG has been used for the correction of *cryptorchidism* in dogs. This practice has a low success rate in boys with congenital cryptorchidism and is considered controversial in all species (Henna et al., 2004; Thorsson et al., 2007). For details see Section Specific Uses of Reproductive Hormonal Drugs.
6) hCG may be used in sequence with eCG for the induction of pseudopregnancy in cats and fertile estrus in dogs (Stornelli et al., 2012).

Adverse reactions and side effects

Immunological reactions such as hives and anaphylaxis due to antihormone antibody production have been reported. Prolonged usage may produce loss of efficacy, which is not reliably correlated with circulating antibody titers in horses (Roser et al., 1979). Use has been associated with prolonged and permanent infertility in cats.

Dose

Veterinary preparations and dosage are listed in Table 27.2.

Equine chorionic gonadotropin (eCG)

Equine chorionic gonadotropin (eCG; formerly known as pregnant mare serum gonadotropin, PMSG) is secreted from the endometrial cups of pregnant mares in early pregnancy in order to induce secondary and accessory corpora lutea (by developing and ovulating additional follicles) and to maintain the primary corpus luteum (and thus progesterone secretion) in the mare. Its gonadotropic activity is primarily FSH-like in the horse and increases ovarian follicular growth, but it has sufficient LH-like activity to induce ovulation or luteinization. Like hCG, eCG is a glycoprotein and nonpituitary gonadotropin with long-lasting biological effects (>24 hours). A single injection is generally sufficient for marked growth of ovarian follicles (Shelton, 1990).

Uses

1) eCG is frequently used to stimulate ovarian follicular growth in the anestrous sheep or goat. It is used in combination with hCG for induction of ovulation and corpus luteum formation.
2) eCG (400 IU) and hCG (200 IU) together make up PG600®, which is used in pigs for inducing follicular growth and estrous cycles in prepubertal gilts (see Section Human Chorionic Gonadotropin).
3) eCG followed by hCG have been used successfully to induce fertile estrus in dogs (Stornelli et al., 2012).

Follicle stimulating hormone (FSH)

Two preparations are available: (i) Folltropin V® (porcine FSH), Vetrepharm, Canada; and (ii) Ovagen® (ovine FSH), ICP Bio, New Zealand. FSH is used to develop multiple follicles in donor cattle for ovulation (superovulation) and oocyte collection, and for production of multiple embryos which may be used in embryo transfer procedures (Hasler, 2002).

Prolactin

Structure and function

Prolactin is synthesized in lactotropes of the anterior pituitary, and is classified as a somatomammotropic hormone because of its structural similarity to GH (somatotropin). It is a 198-amino-acid polypeptide with three intrachain disulfide bridges. Prolactin stimulates lactation in the postpartum period. During pregnancy, prolactin secretion increases and, in concert with other hormones (e.g., estrogen, progesterone), promotes additional mammary development in preparation for milk production (Kooistra and Okkens, 2001). The secretion of prolactin during suckling maintains milk secretion by the mammary gland. It also inhibits ovarian function by suppression of the hypothalamic–pituitary–gonadal axis, and is a component of the regulatory mechanism for seasonality in horses. It is luteotrophic (supports the corpus luteum) in dogs and cats. Prolactin is secreted in a pulsatile manner (half-life, 15–20 minutes).

Mechanism

Prolactin, acting via the prolactin receptor, plays an important role in inducing growth and differentiation of the ductal and lobuloalveolar epithelium; lactation does not occur in the absence of this hormone.

Regulation

Prolactin is unique among the anterior pituitary hormones in that hypothalamic regulation inhibits its secretion. The major regulator of prolactin secretion is *dopamine* (also known as *prolactin inhibiting hormone*, PIH), which is released by tuberoinfundibular neurons and activates the D_2 receptors on lactotropes to inhibit secretion of prolactin. Hence, dopamine agonists or antagonists are expected to affect prolactin release (such as cabergoline and metoclopramide, respectively). Prolactin is the only anterior pituitary hormone for which a unique stimulatory releasing factor has not been identified. Thyrotropin releasing hormone (TRH) can stimulate prolactin release, but its physiological role is unclear. Prolactin is not under feedback control by peripheral hormones.

Therapeutic Uses

Prolactin has no therapeutic uses. Milk production in cattle does not appear to be limited by the circulating prolactin.

Other drugs that affect prolactin

Metoclopramide (Reglan®) is used as an antiemetic in small animals and to stimulate stomach emptying and small intestinal motility in small and large animals via an antagonism of dopamine action. Metoclopramide induces a transient increase of prolactin. Women at risk for developing mammary gland carcinoma are advised to avoid use of metoclopramide, but such a risk factor has not been identified for animals. Efficacy for treatment of agalactia in animals has not been determined, yet. Metoclopramide is discussed in more detail in Chapter 46.

Domperidone (Motilium®, Equidone®) has been available as a tablet for people outside the USA to stimulate gastrointestinal motility and its mechanism of action (also a dopamine antagonist) is similar to metoclopramide. A difference between metoclopramide and domperidone is that the latter does not cross the blood–brain barrier. Therefore, adverse CNS effects with domperidone are not as much of a problem in horses compared with metoclopramide. Fescue toxicosis is caused by a fungus that produces a toxin (an ergot alkaloid = a dopamine agonist) that induces reproductive problems (e.g., decreased lactation or agalactia) associated with decreased prolactin levels in horses. There have been studies to investigate the use of domperidone (which acts to stimulate prolactin secretion via dopamine antagonism) to increase lactation in horses suffering from agalactia associated with fescue toxicity. Equidone oral gel (11%) is FDA approved for prevention of fescue toxicosis and related agalactia in periparturient mares. It is to be administered for 10 days prior to foaling. The recommended dose to be administered to horses is 1.1 mg/kg, daily PO, starting 10 days before the scheduled foaling date. Administration should continue until foaling. If adequate milk production does not occur after foaling, treatment should continue for 5 additional days. Because of drug interactions, domperidone should not be administered with stomach antacids such as omeprazole, cimetidine, or antacids.

Domperidone and sulpiride, another dopamine antagonist, have been used in horses to advance the first ovulation during deep anestrus. In one comparative trial, sulpiride was slightly more effective than domperidone for this purpose (Mari et al., 2009).

Therapeutic uses of drugs that inhibit prolactin are limited primarily to domestic dogs and cats. In these species, unique physiological differences make “antiprolactin” drugs desirable for the purpose of pregnancy termination, treatment of pseudopregnancy, pyometra, and estrus induction. A series of trials have demonstrated that either bromocryptine or cabergoline result in luteolysis and can effectively terminate pregnancy in the second half of pregnancy (Onclin et al., 1995). Use of a dopamine agonist in combination with prostaglandin substantially reduces the dose necessary for clinical efficacy and prevents most side effects resulting from prostaglandin treatment in these species (Onclin et al., 1995). In addition, bromocryptine and cabergoline have been shown to induce fertile estrus cycles in anestrus dogs at least 30 days after the end of diestrus (Verstegen et al., 1999). In a more recent trial, cabergoline was more effective than eCG (Nak et al., 2012).

Oxytocin

Structure

Oxytocin is a cyclic nonapeptide that is structurally similar to vasopressin. It is synthesized as a large precursor molecule in cell bodies of magnocellular neurons located in the paraventricular nucleus (PVN) in the hypothalamus. It is packaged as an oxytocin–neurophysin complex, and secreted from nerve endings that terminate primarily in the posterior pituitary gland. Stimuli for oxytocin secretion include sensory stimuli arising from the cervix and vagina and from suckling of the teats of the mammary gland. Oxytocin is under tonic inhibition (by GABAergic synapses) of the PVN neurons in the hypothalamus. Disinhibition of such control results in rapid firing of oxytocin-containing neurons leading to oxytocin release.

Functions

The two principal targets of oxytocin are the uterus and mammary gland.

Uterus Oxytocin stimulates both the frequency and force of uterine (myometrial smooth muscle) contraction. These effects are highly dependent on estrogen and the immature uterus is quite resistant to the effects of oxytocin. Progesterone antagonizes the stimulant effect of oxytocin, and the decline in progesterone seen in late pregnancy may play an important role in the normal initiation of parturition.

Mammary gland Oxytocin plays an important physiological role in milk ejection. It induces contraction of smooth muscle, most importantly of the myoepithelial cells of the mammary gland, which result in milk ejection. Suckling is the main stimulus that causes oxytocin secretion (Goodman and Grosvenor, 1983).

Oxytocin is also found in birds, fish, and reptiles. In these species, it also has a strong osmoregulatory (antidiuretic hormone or vasopressin-like) effect.

Mechanism of Action

Oxytocin acts via specific G protein-coupled membrane receptors, which upon activation lead to generation of inositol triphosphate (IP_3) from phosphoinositide hydrolysis. IP_3 mobilizes intracellular Ca^{2+} and consequently depolarization–contraction of smooth muscle tissues containing oxytocin receptors.

Clinical Uses

Oxytocin (Pitocin®, Syntocinon® or generic veterinary formulations, e.g., Oxoject®) is primarily used in labor induction and milk letdown (Jeffcott and Rossdale, 1977). Studies also indicate uses in endometritis treatment and for disruption of luteolysis in mares (see below in this section). Oxytocin, originally extracted from animal posterior pituitaries, is now chemically synthesized. The dosage of oxytocin is listed in Table 27.2.

1) Induction of labor: Oxytocin is widely used in small, large, and exotic animal practice to assist parturition (uterine inertia), to induce early or timed parturition, and to encourage postpartum uterine involution and emptying. To induce labor, it is administered intramuscularly or intravenously (Macpherson et al., 1997; Gonzales-Lozano et al., 2009). Oxytocin is used after correction of uterine prolapse in order to promote uterine contractility and involution, preventing reprolapse.
2) Milk letdown. Oxytocin is used empirically to stimulate milk letdown. Oxytocin spray is used intranasally to stimulate milk letdown 5–10 minutes before nursing. However, efficacy of either injectable or intranasal oxytocin has not been established in most domestic species.
3) Persistent postmating endometritis: Oxytocin is a treatment for mating-induced persistent endometritis in the mare; it aids myometrial contractions to clear the uterus of debris following mating (Troedsson, 1999).
4) Inhibition of cyclicity: Evidence has demonstrated that oxytocin administered to mares during early diestrus disrupts the luteolytic event and prevents cyclicity in approximately 60–70% of cases (Vanderwall et al., 2007). Interestingly, the long-acting synthetic analog carbetocin did not have the same clinical effect (Bare et al., 2013). The mechanism of action for the luteal maintenance appears to be unrelated to oxytocin-receptor expression in the uterus, but is associated with decreased prostaglandin production (Keith et al., 2013).

Adverse Reactions and Side Effects

Toxicity is uncommon with oxytocin. Overdose or repeated dosing can lead to painful contractions and even uterine rupture and fetal death.

Prostaglandin Analogs

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is a luteolytic agent produced by the endometrium. In domestic herbivores, it is released in late diestrus in the cycling animal. It is released near term in all pregnant animals (Kindahl et al., 1981). $PGF_{2\alpha}$ mediates a decrease in circulating progesterone via luteolysis (corpus luteum regression) at the end of the cycle and pregnancy. $PGF_{2\alpha}$ acts via the activation of G-protein-coupled receptor linked to IP_3-Ca^{2+}-protein kinase C pathway, which leads to decreased steroidogenesis and luteolysis. There are several prostaglandin analogs (see Tables 27.1 and 27.2) that are used are widely used to regulate estrous cycles, induce abortion, and induce parturition in animals (Schultz and Copeland, 1981).

Uses

1) Estrous synchronization: Dinoprost ($PGF_{2\alpha}$) and its analogs are used for estrous synchronization. They cause premature luteolysis, thus decreasing estrous cycle length and thereby hastening the onset of estrus. It can achieve reliable luteolytic activity after maturation of the corpus luteum is complete (approximately day 5 in horses and ruminants) (Oxender et al., 1975). A study has also demonstrated that $PGF_{2\alpha}$ has "antiluteogenic" properties, and that repeated administration can prevent progesterone from rising after ovulation in the mare (Coffman et al., 2013). Used strategically, in most large animal species except the pig, $PGF_{2\alpha,}$ alone or in conjunction with progesterone/progestin treatment and withdrawal (see Section Progestins) can be used to synchronize estrus in groups of animals. It can also be used in conjunction with GnRH for timed AI or embryo transfer (see Section Gonadorelin).
2) $PGF_{2\alpha}$ is used to induce abortion and parturition in the bitch (Romagnoli et al., 1991). Prostaglandins are effective at inducing luteolysis early in diestrus (Olson et al., 1992; Onclin and Verstegen, 1996). However, the high doses of prostaglandin required in early pregnancy are associated with substantial clinical side effects, including emesis, diarrhea, and cramping. Further, pregnancy may be maintained even after documented luteolysis at this stage (Olson et al., 1992; Feldman et al., 1993). For details for the use of $PGF_{2\alpha}$ to terminate pregnancy see Section Specific Uses of Reproductive Hormonal Drugs.
3) In the cow, $PGF_{2\alpha}$ is used to induce luteolysis and stimulate uterine contractions to facilitate placental delivery (Milvae and Hansel, 1983; Gross et al., 1986).
4) In the mare, $PGF_{2\alpha}$ is frequently used to terminate diestrus and facilitate rapid breeding and to treat mating-induced persistent endometritis. However, studies suggest that prostaglandin use

near ovulation may diminish fertility, calling into question its widespread use (Troedsson et al., 2001; Cuervo-Arango and Newcombe, 2010).

5) In the bitch, $PGF_{2\alpha}$ is used to treat uterine infections via luteolytic and ecbolic effects (see point 2 in this section).

Adverse Reactions and Side Effects

$PGF_{2\alpha}$ causes increased smooth muscle tone, resulting in diarrhea, abdominal discomfort, bronchoconstriction, and increase in blood pressure. In small animals, other side effects include vomiting. Induction of abortion may cause retained placenta.

Dinoprost should not be administered intravenously. It should never be administered to pregnant animals, unless abortion is a desired outcome.

Veterinarians and their assistants should use caution when handling this drug. It should not be handled by pregnant women. Absorption through the skin is possible. People with respiratory problems also should not handle dinoprost.

Reproductive Steroid Hormones (Estrogens, Progesterone, Androgens)

A wide range of steroid hormonal treatment programs have been developed to regulate or induce estrous cycles in small and large animals for breeding purposes. Programs structured to provide increased levels of progesterone, estradiol, and related steroids at the appropriate times, leading to ovulation and pregnancy, have been commonly used in theriogenology (Day, 2004). Androgens are included for discussion in view of their therapeutic uses as anabolic steroid hormones. The three distinct classes of reproductive steroid hormones (estrogens, progestins, and androgens) are discussed here, including their general mechanism of action, pharmaceutical preparations, and therapeutic applications in veterinary medicine.

Mechanism of Steroid Action

The mechanism of action of steroid hormones (estrogens, progestins, and androgens) involves binding to intracellular steroid receptors, which mediate most biological actions of steroids. These steroid receptors are located *inside* the cell, in the nucleus or cytoplasm. Steroid hormones diffuse readily across the cell membrane and bind to intracellular receptors located in the cytoplasm, which are then translocated to the nucleus, or already located in the nucleus. The hormone–receptor complex interacts directly and specifically with sites on the DNA (in the nucleus) called *hormone response elements* (HRE), and act as transcription factors to activate or inhibit gene expression (*transcription*) of the nearby genes. The resulting mRNAs are translated into specific proteins, which brings about a change in the metabolism or other functions of the cell. In contrast to hormones acting through membrane receptors that act rapidly within few minutes or less, the biological effects of steroid hormones generally take longer to occur (hours to days) because of the complex signaling cascade.

A brief description of some important pharmacological steroidal agents used in veterinary practice is given in Table 27.2.

Estrogens

The major ovarian estrogen in females is estradiol. Other estrogens include estrone and estriol, which is produced by the placenta. Estrogens are transported in the blood by binding to sex hormone-binding globulin (SHBG). Estradiol is active by the oral route for pharmacotherapy, but has low bioavailability because of extensive first-pass hepatic metabolism. Therefore, for some treatments injectable preparations are used. For oral administration, semisynthetic derivatives (ethinyl estradiol) or synthetic estrogens (mestranol) or nonsteroidal derivatives (diethyl stilbosterol, DES) have been developed for clinical use.

Therapeutic Uses

Estrogen is essential for normal development of secondary sexual phenotype and reproductive behavior in females. Estrogen modifies serum protein levels and reduces bone resorption. It is also an effective feedback suppressor of pituitary LH and FSH secretion. Estrogens may be useful in the treatment of the following conditions: hypogonadism; postmenopausal symptoms (humans); contraception/ elective pregnancy termination; persistent vaginitis in prepubertally spayed bitches; benign prostatic hyperplasia/ carcinoma of prostate (canine).

Adverse reactions and side effects

The most common toxicity in humans associated with chronic estrogen usage includes breast cancer, endometrial hyperplasia, breakthrough bleeding, and uterine carcinoma. Toxicity recognized in domestic carnivores includes bone marrow suppression, increased risk of pyometra, tendon laxity, and ventral alopecia.

Veterinary Uses

Estrogens are not widely used in veterinary medicine, especially in food animals due to the concerns about drug residues in meat for human consumption. The following estrogen preparations have been used in veterinary medicine. *Estradiol benzoate* and other estrogens have been used for the purpose of estrus induction and to prevent implantation of embryos in bitches (Kutzler, 2005; Sutton et al., 1997). In cattle, they have been suggested for the treatment of postpartum cow to decrease uterine infection and to hasten uterine involution.

Diethylstilbestrol (DES, Stilboestrol®) is a nonsteroidal estrogenic agent used in the management of estrogen-responsive urinary incontinence and persistent vaginitis in spayed animals, and for estrus induction and to prevent implantation of embryos in bitches. It has been used in the treatment of benign prostatic hyperplasia and perianal gland adenoma in dogs (Kutzler, 2005; Hill et al., 2012; Sutton et al., 1997; Niżański, 2014). *It should be noted that the use of estrogens for many of these purposes is controversial and that better alternatives may be available.*

Progestins

Progesterone is the major progestin in humans and animals. Progesterone is rapidly metabolized in the liver and therefore has a very low bioavailability and a short half-life. Synthetic progestins altrenogest (allyl-trenbolone, Regumate®) and melengestrol acetate (MGA) are widely used in veterinary practice to induce long-lasting ovarian suppression and synchronization of estrous cycle following progestin withdrawal in small and large animals. Progestins act on progesterone receptors in the hypothalamus/ pituitary, increasing the negative feedback and decreasing FSH and LH output. The withdrawal of progestin therapy initiates a new estrous cycle and ovulation.

Adverse Reactions, Side Effects, and Precautions

Humans handling progestin formulations should wear gloves and avoid contact, as for example altrenogest can be absorbed through intact skin. Contraindications for the use of progestins can be a history of endometritis or pyometra. There might also be an increased risk for diabetes mellitus.

The following preparations of natural progesterone or synthetic progestins are most widely used in veterinary medicine.

Altrenogest

Altrenogest (Regumate®), also known as *allyl-trenbolone*, is a synthetic, orally active progestin indicated for controlling the estrous cycle of the mare and pig (see Figure 27.1).

Altrenogest is indicated to suppress estrus in mares to allow a more predictable occurrence of estrus following withdrawal of the drug (Davis et al., 1985; Shimatsu et al., 2004). Altrenogest (0.044 mg/kg, PO) is recommended for 15 days to suppress estrus and allow for a predictable occurrence of estrus in mares. This effect is enhanced by administration of both a progestin and an estrogen. In addition, altrenogest and other progestins have been used to advance the first ovulation of the breeding season. However, the use of Regumate is controversial and some studies suggest altrenogest alone has a limited ability to shorten the transition period. Altrenogest is not effective during winter anestrus. Ovulation occurs 9–11 days after altrenogest therapy. Use of a prostaglandin ($PGF_{2\alpha}$) immediately following altrenogest withdrawal will induce estrus. Generally, altrenogest may be helpful to control the equine estrous cycle, but it has not always been able to exert predictable control over the estrous cycle of the mare (Lofstedt and Patel, 1989). Altrenogest can also be used in synchronizing estrus in the gilt (Davis et al., 1985). Swine producers are interested in synchronizing estrus in gilts to reduce the number of replacement gilts and for optimizing breeding by AI. Altrenogest is given to gilts at 15–20 mg daily for 18 days, which results in 95% expression of estrus 4–7 days after altrenogest withdrawal. Research has demonstrated that altrenogest administration may increase litter size in a subpopulation of sows (van Leeuwen et al., 2011).

In addition, altrenogest is widely used in mares for the prevention of pregnancy loss (Daels et al., 1991, 1996). A "double-dose" of altrenogest (0.088 mg/kg) can be used in pregnancy to minimize the abortion in mares suspected of hypoluteoidism or which experience either prostaglandin or endotoxin exposure, or for mares diagnosed with placentitis (Bailey et al., 2010). Although altrenogest is considered safe in pregnant mares, some research suggests it may be associated with fetal or neonatal abnormalities, or with slight delays in parturition (Neuhauser et al., 2009).

Melengestrol Acetate

Melengestrol acetate (MGA) is a progestin based, feed-additive system to synchronize estrous in cattle. This product is indicated for use in estrus suppression in feedlot heifers and has been used with increased frequency to synchronize breeding females. MGA can be fed at a rate of 0.5 mg per head per day to suppress estrus. Once MGA is removed from the feed, females will begin cycling within a few (normally 2 to 3) days. Briefly, the MGA program consists of providing MGA at the prescribed level in a feed product the cattle will consume, once daily for 11 to 14 days. After removal of the MGA from the feed, estrus follows after 48 to 72 hours. At this point, breeding can begin with bulls or via artificial insemination. MGA is a simple treatment, but is generally less efficient than other estrous synchronization products.

CIDR® – Progesterone-Containing Controlled Internal Drug Release Intravaginal Device

Several CIDR (Controlled Internal Drug Release) intravaginal progesterone-releasing devices are marketed for

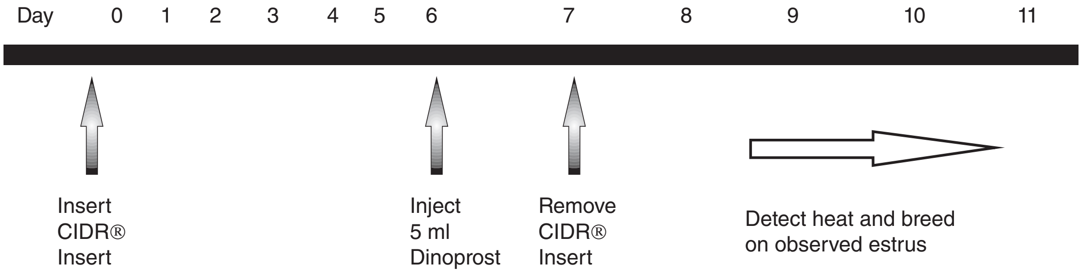

Figure 27.2 Eazi-Bred^{TM} CIDR protocol for synchronization of estrus in cattle. The Eazi-Breed CIDR for cattle consists of 1.38 g progesterone releasing device for vaginal insertion in breeding programs. CIDR, controlled internal drug release.

cattle (Eazi-Breed), sheep (CIDR-S^{TM}), and goats (CIDR-G^{TM}). The main objective of these products is synchronization of estrus, to improve the timing and efficiency of breeding programs of both dairy and beef breeds of cattle (Yavas and Walton, 2000; Lamb et al., 2010; Wiltbank et al., 2011). The Eazi-Breed CIDR contains 1.38 g progesterone in silicon molded over a nylon spine. These inserts are to be administered intravaginally, one per animal, in dairy heifers for breeding. It releases progesterone during the 7-day treatment period (Figure 27.2). To induce definitive synchronization, an injection of a prostaglandin dinoprost tromethamine (Lutalyse®) is given to all heifers one day before insert removal. Removal of the insert on treatment day 7 results in a decline in plasma progesterone, triggering estrus within 3 days.

Androgens and Finasteride

Testosterone

Testosterone is the principal circulating androgen in males. It is secreted by the Leydig cells of the testes in response to LH from the pituitary gland. Testosterone regulates the male reproductive system by binding to the androgen receptor present especially in reproductive tissues, muscle, and fat. It is particularly responsible for normal male sexual differentiation. Some physiological effects of testosterone are mediated by conversion to dihydrotestosterone, a more potent androgen than testosterone (Figure 27.3). Testosterone also is converted to estradiol by Sertoli cells of testis in the adult. Several analogs of testosterone that possess greater *anabolic* (protein building) than *androgenic* (male sexual) effects have been synthesized, which are known as *anabolic steroids***.** These include *nandrolone, stanazolol, oxymethalone,* and others. The major indication for the use of anabolic steroids is in equine medicine to increase athletic performance. Anabolic steroids increase muscle size, strength, and increase red blood cell production. Excretion of urea nitrogen is also reduced ("positive nitrogen balance"). Many of the anabolic steroids are tightly controlled in horses involved in competition, and care should be exercised to ensure that their administration falls within the guidelines of the governing organization in question (Soma, 2007, 2008). Furthermore, administration of androgens or anabolic steroids should be performed with caution in potential breeding animals due to the known negative feedback effects on the ovarian and spermatogenic cycles.

Therapeutic uses There are only few clinical evaluations of testosterone for therapeutic use in veterinary medicine.

Anabolic Steroids

Anabolic steroids are controlled substances that predominantly produce greater anabolic than androgenic effects, and, as described in Section Stanazolol, Nandrolone, Boldenone Undecylenate, Trenbolone Acetate, are used in equine practice to increase athletic performance. In addition, these agents have been used in the treatment of aplastic anemia (dogs and cats), myeloproliferative disease, and lymphoma accompanied by nonregenerative anemia. They increase myelopoiesis, stimulate the production of erythropoietin, and increase

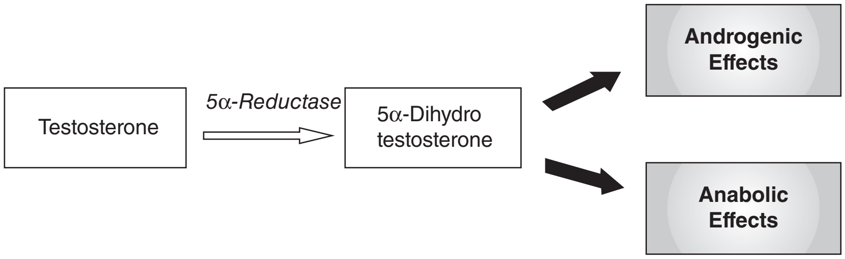

Figure 27.3 Metabolism and major effects of testosterone. Testosterone is converted in several organs to dihydrotestosterone (DHT), which is the active hormone in those tissues. Testosterone and DHT produce both androgenic and anabolic actions.

production of erythrocytes. They stimulate appetite, promote a positive nitrogen balance, and muscle calcium levels (Brower, 1993). Several weeks to months may be required for a positive response to be seen, and even with this prolonged therapy only one-third of dogs and cats show a positive erythropoietic response to anabolic steroids. Anabolic steroids bind to androgen receptors present especially in reproductive tissue, muscle, and fat. The ratio of anabolic ("body-building") effects to androgenic ("virilizing") effects may differ among the members of this class of drugs, but in practice all agents possess both properties to some degree (Figure 27.3).

Stanazolol, Nandrolone, Boldenone Undecylenate, Trenbolone Acetate

Stanazolol (Winstrol-V$^{®}$) is an anabolic steroid with strong anabolic and weak androgenic activity. It is potentially useful as an adjunct to the management of catabolic disease states (Cowan et al., 1997). It has been recommended to stimulate erythropoiesis, arouse appetite, promote weight gain, and increase strength and vitality. However, the efficacy of stanazolol is controversial and requires 3–6 months' treatment before a positive change may be seen. Stanazolol is most commonly used in horses to enhance athletic performance. Stanazolol is potentially hepatotoxic. Stanazolol may produce weight gain, sodium and water retention and, exacerbate azotemia. It may promote hypercalcemia, hyperphosphatemia, and hyperkalemia.

Nandrolone (also referred to as 19-nortestosterone) is also a potent synthetic anabolic androgenic steroid. Nandrolone is useful in the treatment of certain rare forms of aplastic anemia, which are or may be responsive to anabolic androgens. It has been used in some cases to counteract catabolic states, for example after major trauma or strenuous physical exercise (Hyyppa, 2001).

Boldenone (Equipoise$^{®}$) is a steroid ester anabolic agent. It is used, primarily in horses, to improve nitrogen balance, reduce overexertion associated with exercise, and improve training. It may also improve appetite and improve weight gain when used with a well-balanced diet. Boldenone is a long-lasting agent and effects may persist for 6 weeks after an intramuscular injection.

Trenbolone acetate is not used therapeutically, but is used as an implant (often combined with estradiol) approved for use as a growth promoter for beef cattle. Although these implants have an important economic benefit to the beef cattle industry (Duckett and Pratt, 2014), research indicates a possible risk of trenbolone acetate as an endocrine disrupting substance in the environment (Qu et al., 2013).

Adverse Reactions and Side Effects

After administration of androgens increased masculine effects and possibly aggressiveness are common. In human, the use of some anabolic steroids is associated with hepatic toxicity.

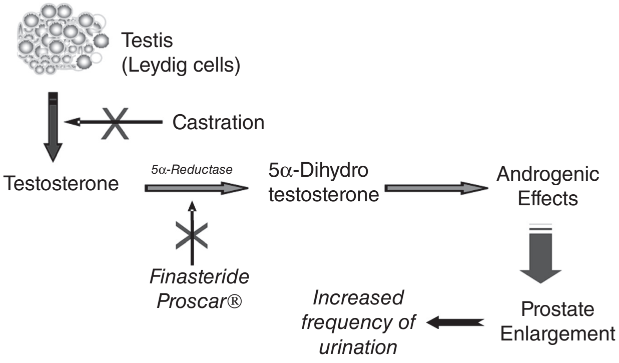

Figure 27.4 Treatment options for benign prostatic hypertrophy. Reduction of androgen effects is an important therapeutic goal for benign prostatic hypertrophy (BPH). Inhibition of dihydrotestosterone produced by finasteride is a key pharmacotherapy in dogs affected by BPH.

Anabolic steroids are contraindicated in dogs with benign prostatic hypertrophy. Anabolic steroids may produce carcinogenicity and teratogenicity.

Finasteride

Finasteride (Proscar$^{®}$) is an irreversible 5α-reductase inhibitor that blocks the conversion of testosterone to 5α-dihydrotestosterone, a potent androgen receptor agonist (Figure 27.4). Testosterone itself interacts weakly with the androgen receptor. Therefore, most of the biological effects of testosterone have been attributed to the synthesis of endogenous 5α-dihydrotestosterone. In the prostate gland, 5α-dihydrotestosterone has been implicated to cause benign prostatic hypertrophy.

Uses

Finasteride is the drug of choice for benign prostatic hypertrophy in dogs. Finasteride induces prostatic involution by apoptosis in dogs with spontaneous benign prostatic hypertrophy (Sirinarumitr et al., 2002). Finasteride is highly effective in benign prostatic hypertrophy cases. Finasteride has not proven valuable in prostatic carcinoma.

Dose

Finasteride (0.1 to 0.5 mg/kg, PO once daily) therapy for 16 weeks may reduce prostate size in canine prostatic hypertrophy.

Adverse Reactions and Side Effects

The major potential side effect is *impotence*. Finasteride, however, did not affect semen quality or serum testosterone concentrations in experimental studies in dogs.

Specific Uses of Reproductive Hormonal Drugs

Superovulation

Cattle may be induced to ovulate multiple follicles (superovulation) so that embryos can be transferred to multiple recipient animals to increase the number of progeny. In cattle, superovulation results in about 10 ovulations, compared to the normal, single ovulation. Superovulation, on the average, results in about six usable embryos. The ideal response is five to 12 embryos from one-third of the donors. The superovulation injection program of donor cows is initiated between day 9 and 14 of the estrous cycle, with FSH given over 4 days and $PGF_{2\alpha}$ given on days 3 and 4 of treatment. In the case of goats and sheep, the drug regimen for superovulation depends upon the reproductive status. During the breeding season, $PGF_{2\alpha}$ is used to mediate luteolysis, and gonadotropins are given to increase the ovulatory rate. Dairy goats were superovulated successfully during the breeding season by placement of an intravaginal sponge (medroxyprogesterone acetate, 60 mg) for 11 days, 125 μg cloprostenol IM on days 1 and 9 of sponge treatment, and twice daily injections of FSH-P (2.5 mg) IM for 3 days beginning on day 9 of sponge treatment (Thompson, 2001). The anestrous animal is given a progestin followed by gonadotropin treatment.

Pseudopregnancy

For treatment of queen (female cat) in heat, animals may be treated with GnRH or hCG. These animals are induced ovulators and in the absence of the mating stimulus, ovulation and corpus luteum formation must be induced with GnRH or hCG, as described in Section Gonadotropin-Releasing Hormone, Gonadorelin, and Gonadotropins, to prevent repeated estrus periods. In some rodents (rats and mice), a state of pseudopregnancy associated with prolonged high serum progesterone levels can be induced by sequential administration of eCG and hCG elevated progesterone (Reddy et al., 2001). This sequential regimen may be useful in transgenic mice breeding programs.

In contrast, in dogs pseudopregnancy refers to inappropriate mammary development and mothering behavior in nonpregnant bitches. This condition is related to the normal physiology of the bitch and can often be managed conservatively by increasing the animal's activity and reducing stimuli that encourage maternal behavior. However, recent data have suggested that bitches experiencing repeated and severe pseudopregnancy may be at increased risk for pyometra or mammary tumors. Clinical resolution of pseudopregnancy can be achieved by administration of a dopamine agonist such as cabergoline during late diestrus.

Parturition

Parturition may be induced as an elective procedure in many species. The reasons for induced parturition include: (i) enhancing the ability of managers to attend parturition, (ii) attempting to save either the neonate or dam in cases of severe illness, and (iii) synchronize parturition for production purposes.

In cattle, dexamethasone (20–30 mg) or flumethasone (8–10 mg) IM is 80–90% effective in parturition induction, with calving occurring 24–72 hours posttreatment. Calving may be induced with $PGF_{2\alpha}$ (25–30 mg) or cloprostenol (500 μg) IM with results similar to that found with glucocorticoids. The interval from injection to calving is 24–72 hours. A combination of treatments with dexamethasone and cloprostenol has resulted in a shorter interval from treatment to delivery and a greater percentage induced compared to either drug alone or two injections of cloprostenol. The optimal farrowing response to $PGF_{2\alpha}$ is achieved by administration within 2 days of normal delivery for a herd. Induction of parturition in the mare is considered controversial and associated with higher complication rates than other large animal species. A combination of prostaglandin E and sequential doses of 15 IU of oxytocin increased success rates, compared to oxytocin alone (Rigby et al., 1998). Subsequently, low doses of oxytocin (2.5–3.5 IU) have been shown to be safer. A small dose of oxytocin given once daily fails to induce parturition until after fetal maturation, while resulting in foaling within 120 minutes in 68–95% of mares with mature foals (Camillo et al., 2000; Villani and Romano, 2008). Alternately, precocious fetal maturation has been successfully induced using dexamethasone (100 mg q 24 h for 3 days; Ousey, 2011).

Termination of Pregnancy (Abortion)

Pregnancy termination is one of the most common "reproductive" requests from dog and cat owners because of mismating or other reasons. Pharmacological agents are available that can prevent or terminate pregnancy (Eilts, 2002). Estrogens can be given during estrus to prevent pregnancy (Sutton et al., 1997); however, their use is controversial and should be avoided if possible. A pregnancy examination should be performed before any drug is given to terminate pregnancy. If a dog is known to be pregnant, multiple doses of natural prostaglandins (25–250 μg/kg) or synthetic prostaglandins (cloprostenol, 1–5 μg/kg) can be used throughout pregnancy. However, higher doses of prostaglandins are associated with substantial clinical side effects, including vomiting, diarrhea, lethargy, and cramping. Thus hospitalization and careful monitoring of animals are recommended to control side effects, which tend to be more severe early and diminish during the course of treatment. Side effects may be minimized by using low doses after day 30 (30–50 μg/kg, three to four times daily for 4–11 days) (Olson et al., 1992), or by starting with a lower dose and gradually increasing it (30–50 μg/kg, increased to 100–200 μg/kg). Additional complications associated with prostaglandin use for abortion are incomplete luteolysis and treatment failure or mummification of dead pups/kittens. Multiple doses of inhibitors of prolactin secretion (cabergoline, bromocriptine) or dexamethasone are associated with fewer side effects, but are not effective in the first half of gestation. Combined protocols of prostaglandin and prolactin inhibitors are also effective at terminating pregnancy beginning day 25 post-LH (Onclin et al., 1995). Progesterone blockers such as mifepristone and aglepristone are effective, but not available in the USA. Other drugs, such as the isoquinolones and progesterone synthesis inhibitor epostane, are available outside of the United States and appear to be very effective at terminating pregnancy.

Cryptorchidism

Cryptorchidism (dog) is the failure of one or both testes to be present in the scrotum by 6–8 weeks of age. The testes migrate from the caudal pole of the kidney to the scrotum via guidance from the gubernaculum testis. At birth, the testes in the dog are usually within the abdomen near the internal inguinal ring. The testes move through the inguinal canal and are in the scrotum by 10–14 days of age. Because this condition can have a genetic basis, dogs with this condition should not be used for breeding purposes. The most common method of treatment is a series of injections of hCG. Dogs younger than 16 weeks of age are the best candidates. The IM injection of 100–1000 IU hCG four times in a 2-week period is expected to result in testicular descent in a majority of dogs.

However, success rates are variable and cryptorchidism is typically not treated and they are not encouraged to enter the genetic pool (Goericke-Pesch, 2010). Likewise, in other species (cattle, sheep, goats, horses), pharmacological treatment of cryptorchidism is generally not supported.

Benign Prostatic Hypertrophy

Benign prostatic hypertrophy (BPH) is a spontaneous and age-related disorder of intact male dogs, which occurs in more than 80% male dogs over 5 years of age. BPH is associated with clinical signs of sanguineous prostatic fluid, constipation, and dysuria. BPH is linked to the androgenic activity of dihydrotestosterone, metabolized locally from testosterone, on accessory sex glands (enlargement of prostate gland). BPH can be identified by the signs such as increased frequency of urination, constipation, or blood in urine. BPH often occurs concurrently with prostatic infection, abscessation, cysts, and neoplasia in the intact dog. The treatment of choice is castration or antiandrogenic agents such as finasteride, a 5α-reductase inhibitor, or flutamide, an androgen receptor antagonist (Iguer-Ouada and Verstegen, 1997; Johnston et al., 2000). Occasionally, progestins are used. However, these are associated with decreased libido and fertility (Niżański et al., 2014).

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28

Thyroid Hormone and Antithyroid Drugs

Duncan C. Ferguson

Introduction

Hypothyroidism

Hypothyroidism is the most common endocrinopathy of the dog as a result of the breed-associated incidence of lymphocytic thyroiditis. Clear syndromes of congenital hypothyroidism are recognized in the neonatal horse, but the incidence and pathogenesis in the adult horse is less understood. Spontaneous hypothyroidism in the cat and other domestic species is most commonly associated with congenital disease or following thyroidectomy. Less commonly, hypothyroidism can be caused by iodine deficiency or by the ingestion of goitrogenic substances in the environment or food; goitrogenic substances are compounds that interfere with thyroid hormone synthesis by the thyroid gland.

Clinical signs of hypothyroidism common to most species generally reflect the reduction in basal metabolic rate of the body and include lethargy, mental depression, weakness, inability to train, and/or nonpruritic hair loss (Ferguson, 1989a, 1993; Ferguson and Hoenig, 1991b; Ferguson et al., 1992; Peterson and Ferguson, 1990; Feldman and Nelson, 2004a).

Hyperthyroidism

Hyperthyroidism is now the most common endocrine disorder in the cat, and is only occasionally seen in other domestic species. Hyperthyroidism or thyrotoxicosis is caused by excessive concentrations of the circulating thyroid hormones, thyroxine (T_4) and triiodothyronine (T_3), most commonly the result of hyperplastic or benign adenomatous malignant thyroid glands in cats and adenocarcinomas in dogs. Hyperthyroidism occurs most frequently in middle-aged to geriatric cats and discussion of therapeutic agents will focus on those used in this species. The most common clinical signs associated with hyperthyroidism, which can be directly related to thyroid hormone excess, are weight loss in spite of ravenous appetite, hyperactivity, polydipsia, polyuria, diarrhea, intermittent fever, vomiting, and symptoms of cardiovascular disease such as tachycardia and dyspnea. Often the cats shed excessive amounts of hair or the coat may be matted. Rarely, hyperthyroid cats present in a way similar to what has been called "apathetic hyperthyroidism"; the cats are lethargic and often anorectic, perhaps representing an end-stage form of the disease (Feldman and Nelson, 2004b; Ferguson and Hoenig, 1991a; Peterson and Ferguson, 1990). Therapy focuses upon either definitive treatments such as radioiodide and thyroidectomy, or, as discussed below, the administration of drugs that reduce the synthesis, secretion, metabolism, and/or action of thyroid hormone.

Thyroid Physiology

Iodine Metabolism

Thyroid hormones are the only iodinated organic compounds in the body with the two major secretory products of the thyroid gland, thyroxine ($\text{L-}T_4$) and 3,5,3′-triiodothyronine ($\text{L-}T_3$) containing 65% and 59% iodine, respectively. The minimum iodine requirement of most animals is unknown, but the daily amount needed in the ration to prevent goiter in all animals is generally accepted to be about 1 μg/kg body weight. The daily recommended amount of iodine in the dog is 15 μg/kg and while it has not been carefully studied in the cat, it is believed to be about 100 μg/cat/day. Commercial cat foods have been shown to be quite variable in the amount of iodide provided, probably a result of the variable amount of seafood used to create the diet. Indeed, dietary iodide has been shown to be inversely related to free T_4 concentrations (Tarttellin and Ford, 1994). Also, a review has questioned the adequacy of iodide in commercial cat diets before 2006, suggesting that the increased incidence of feline hyperthyroidism might have been associated with iodide deficiency (Edinboro et al., 2010). Although true nutrient requirements for this micronutrient are not well established, most commercial dog and

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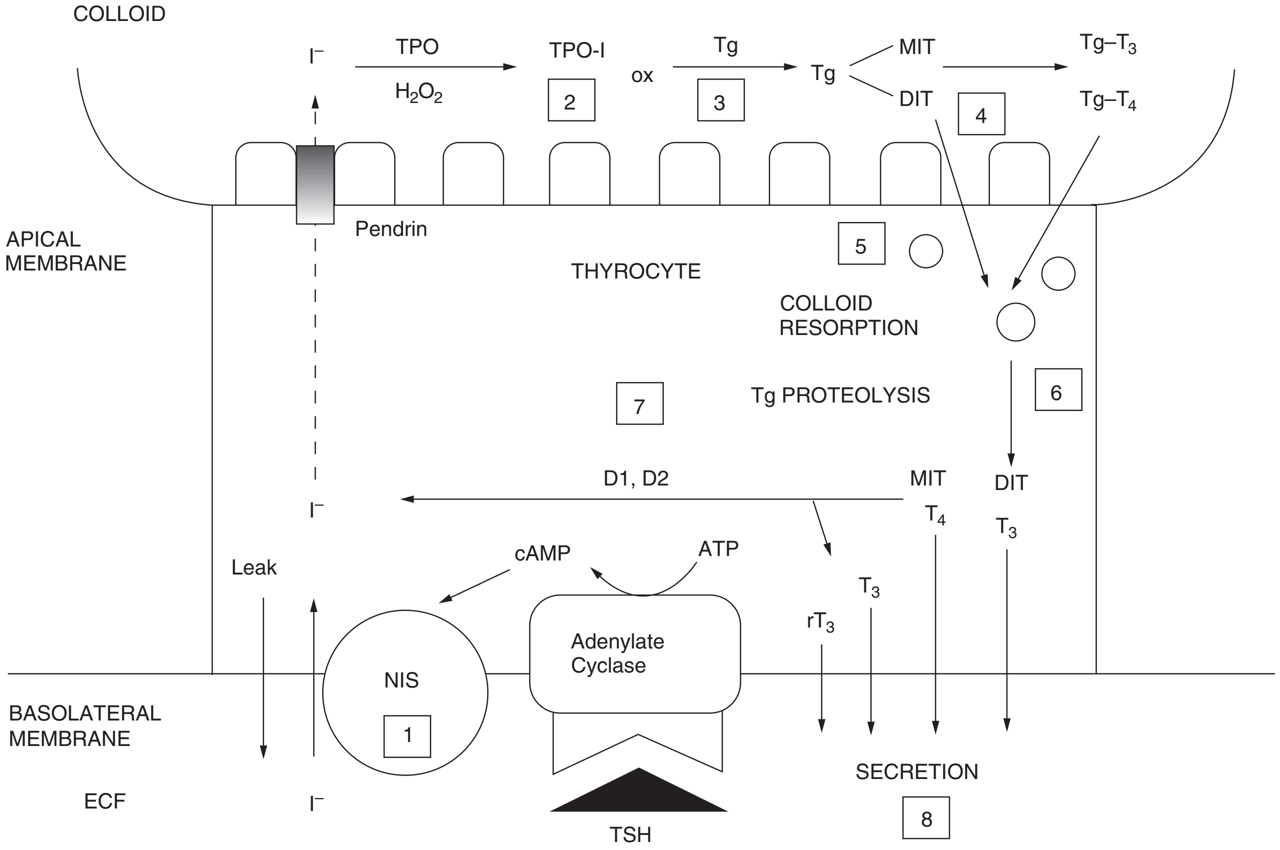


Figure 28.1 Thyrotropin (thyroid-stimulating hormone, TSH)-stimulated iodide uptake and organification by the thyrocyte. **Step 1:** Inorganic iodide (I^-) is actively translocated by the sodium–iodide symporter (NIS), using the Na^+ gradient established by the Na^+K^+-ATPase. NIS is stimulated directly by the G protein-coupled TSH receptor, and is influenced by availability of iodide, being stimulated by iodide insufficiency and suppressed by excess iodide. After diffusing to the apical or brush border membrane, the pendrin protein carries it into the lumen of the colloid. **Steps 2 and 3 Oxidation and organification:** Iodide is oxidized by the thyroid peroxidase enzyme (TPO) (Step 2) and added to a tyrosine residue of preformed thyroglobulin (Tg) (Step 3) to form monoiodotyrosine (MIT) and diiodotyrosin (DIT). **Step 4 Coupling:** The MIT and DIT residues on Tg couple to form T_3, and two DIT residues couple to form T_4. Steps 2–4 are sensitive to thionamide antithyroid drugs like PTU or MMI. **Step 5 Colloid resorption:** Under the influence of TSH, follicular colloid–containing Tg is resorbed by the thyrocyte by pinocytosis. **Step 6 Tg proteolysis:** Thyroid hormones MIT and DIT are released from Tg under the influence of TSH. **Step 7 Deiodination:** Also stimulated by TSH at the time of secretion, deiodinase enzymes (D1,D2) are stimulated, resulting in the removal of iodide and increasing the T_3/T_4 ratio in the secreted pool. **Step 8 Secretion:** T_4, T_3, and rT_3 are released into the bloodstream. Source: Peterson and Ferguson, 1990. Reproduced with permission of Elsevier.

cat food preparations include at least three to five times the minimum stated requirement for iodine when fed in recommended amounts. As a result, today, documented iodine deficiency has become a rare condition in most domestic animals. During pregnancy, the recommended minimum daily requirement for iodine is increases fourfold. Areas of iodine deficiency in North America include the Great Lakes region and eastern British Columbia (Kaptein et al., 1994; Peterson and Ferguson, 1990; Zimmerman, 2013).

Ingested iodine is converted to iodide in the gastrointestinal tract and absorbed into the circulation. The dog has plasma iodide concentrations of 5 to 10 μg/dl, which are 10 to 20 times the levels in human plasma. In the thyroid gland, iodide is concentrated or "trapped" by the sodium–iodide symporter (NIS), which utilizes the sodium gradient developed by the Na^+,K^+-ATPase to move iodide through the basolateral plasma membrane of the thyroid follicular cell. This results in intracellular iodide concentrations that are 10 to 200 times that of serum. This process is stimulated by the interaction of thyrotropin (TSH) with G-protein-coupled TSH receptors on the surface of the follicular cell leading to the stimulation of cAMP (Figure 28.1). The transcription of NIS is stimulated by TSH or cyclic AMP. NIS is also upregulated directly by thyroidal mechanisms reacting to iodide insufficiency or blockade of iodide transport by compounds such as perchlorate. Other tissues, including the salivary glands, gastric mucosal cells, renal proximal tubule cells, placenta, ciliary body, choroid plexus, and mammary glands, can take up considerable amounts of radioiodide in a TSH-independent fashion.

Diagnostically, radioactive iodide or pertechnetate (TcO_4^-), which, unlike iodine, cannot be organified, can be used to assess the anion transport function (uptake) by the thyroid gland. Iodide trapping can be inhibited by other anions such as thiocyanate (SCN^-), NO_3^-, and ClO_4^-. Thiocyanate is a metabolic product of some naturally occurring compounds in plants and may result in goitrogenic (antithyroid) activity of the plant. Domestic animals may also be exposed to thiocyanate produced as a by-product of cigarette smoke in a household environment. Oral administration of perchlorate following the administration of a tracer dose of radioiodine can be used to diagnose congenital defects in the thyroidal organification of iodide (perchlorate discharge test) (Taurog, 1991; Kopp, 2013).

Thyroid Hormone Synthesis

Thyroglobulin (Tg), an iodinated glycoprotein with a molecular weight of 660,000 daltons, serves as a synthesis and storage site for thyroid hormone and its precursors in the thyroid follicle. After synthesis within the endoplasmic reticulum of the thyroid follicular cell, membrane vesicles containing noniodinated Tg fuse with the apical membrane and are released (by exocytosis) into the follicullar cell lumen where thyroglobulin is stored as colloid. Once inside the thyroid cell, iodide is transported to the follicular lumen by the protein pendrin, then oxidized by the enzyme thyroid peroxidase (TPO) to iodine (Figure 28.1). Pendrin is an apical transmembrane protein that transports sulfate, chloride, iodide, and bicarbonate (Larsen et al., 2003). Iodide is then incorporated by the heme protein enzyme thyroid peroxidase (TPO), which, in the presence of hydrogen peroxide, adds iodide onto tyrosine residues of Tg in a process called *organification,* forming monoiodotyrosine (MIT) and diiodotyrosine (DIT). TPO also forms thyroxine (T_4) by coupling two DIT molecules, and 3,5,3′-triiodothyronine (T_3) by coupling one MIT molecule with one DIT molecule (Burrow et al., 1989; Peterson and Ferguson, 1990; Taurog, 1991; Kopp, 2013). The reaction catalyzed by TPO is inhibited by the thionamide drugs propylthiouracil (PTU) and methimazole (MMI) and by high concentrations of iodide (the *Wolff–Chaikoff effect*). The intermediate product of peroxidation of iodide is either hypoiodous acid (HIO_2^-) or iodinium (I^+). The H_2O_2 required is generated by NADPH oxidase. In normal humans and rats, more than 90% of thyroidal radioiodine is organified to iodotyrosines and iodothyronines within minutes of entry into the thyroid.

When iodine intake is adequate, production of T_4 is favored. However, in iodine-deficient states and impending thyroid failure, the intrathyroidal synthesis of T_3 is preferred over that of T_4. By this autoregulation, the thyroid gland produces the most active thyroid hormone (T_3 is three to ten times more potent than T_4) while using less iodide. Conversely, chronic iodine excess may lead to excessive storage of thyroidal hormone.

The Wolff–Chaikoff effect, another intrathyroidal regulatory mechanism, is key to understanding the potential acute antithyroid effect of large amounts of ingested iodide. It is believed that, through this mechanism, the organism is protected from massive thyroid hormone release following a large dietary iodine load (Taurog, 1991; Wolff, 1989). Although the acute Wolff–Chaikoff effect is still not well understood, iodine itself has been shown to inhibit Ca^{++} (and NADPH-dependent H_2O_2) generating activity associated with NADPH oxidase, a mechanism characterized in dog, pig, and human thyroid glands. It has been proposed to be associated with autoregulation by organic iodinated compounds in the thyroid. Furthermore, this enzyme seems to be inhibited by iodinated compounds in cultured dog thyroid cells (Dugrillon, 1996; Panneels et al., 1994). In humans, the Wolff–Chaikoff effect is transient and “escape” is seen within several weeks and has been proposed to be associated with reduced iodide transport into the gland (Braverman and Ingbar, 1963).

Thyroid Hormone Secretion

Thyroid hormone secretion is initiated as the epithelial follicular cells take up thyroglobulin in colloid droplets by a process called *pinocytosis*. Simultaneously, lysosomes (containing proteases and hydrolytic enzymes) migrate from the basal region of the cell and fuse with the colloid droplets (Figure 28.1). Degradation of thyroglobulin by the lysosomal proteolytic enzymes produces both the iodotyrosines (MIT and DIT) and iodothyronines (T_4 and T_3). Little of the released MIT and DIT enters the circulation because the iodine is removed from these compounds by deiodinase enzymes (Figure 28.1). Some of this iodine is recycled internally for iodination of new tyrosine residues in thyroglobulin, but in carnivores much iodine is released to the circulation (Belshaw et al., 1974; Kaptein et al., 1994). This inefficient thyroidal reutilization of iodine may help explain the high daily iodine requirements of the dog and cat compared to humans.

Proteolysis of Tg, as described in Section Thyroid Hormone Synthesis, liberates relatively large amounts of T_4, but only small quantities of T_3, into the cytosol. 5’-deiodinase enzymes (D1 and/or D2) within the thyroid gland, however, can deiodinate T_4 to either T_3 or 3′,5′,3-T_3 (reverse T_3) and less-iodinated products. As a result, although the $T_4 : T_3$ ratio stored in the gland is 12 in the canine thyroid, the ratio of secreted products is 4. Production rates of the thyroid hormones in the dog have been estimated to be 8 μg/kg/day for T_4 and 0.8 to 1.5 μg/kg/day for T_3. In cats, T_4 production rates have been estimated to be 5.6 µg/kg/day, and 0.4 µg/kg/day for T_3. These production rates are greater than twice those for T_4 and greater than three times the rate for T_3 in humans (Kaptein et al., 1993, 1994).

Hypothalamic–Pituitary–Thyroid Extrathyroid Axis

Thyrotropin (thyroid-stimulating hormone; TSH), a glycoprotein produced in the thyrotropes of the pituitary pars distalis, has a stimulatory effect on thyroid hormone synthesis and secretion. In addition, TSH stimulates thyroid growth, probably in conjunction with actions of the insulin-like growth factors (IGF I and II). Thyrotropin has a molecular weight of about 30,000, consisting of an α subunit (identical to the α subunit of the other glycoprotein pituitary hormones luteinizing hormone, LH, and follicle-stimulating hormone, FSH), and a β subunit, which is specific to the TSH molecule (see Chapter 26). TSH binds to a specific TSH receptor on the thyroid follicular cell membrane and stimulates adenylate cyclase, the production of cyclic AMP, and the active uptake of inorganic iodide (Figure 28.1). The TSH receptors in dog, cat, and humans have been cloned and expressed. TSH also stimulates the synthesis of thyroglobulin, its release into the colloid, and its iodination by TPO (i.e., organification). As a final step in the delivery of hormone into plasma, TSH stimulates thyroglobulin resorption and proteolysis to release T_3 and T_4. The thyroidal deiodinases are also stimulated by TSH (Magner, 1990; Rapaport and Nagayama, 1992; Shupnick et al., 1989).

A detailed study of the hypothalamic–pituitary–thyroid–extrathyroid axis is only possible with the availability of a valid TSH immunoassays for each species. Availability of this assay for the dog has helped to demonstrate that most of the replacement dosages of L-thyroxine recommended are well in excess of those needed for suppression of endogenous TSH into the normal range. The currently available assays do not reliably distinguish between normal and low TSH concentrations making it more difficult to confirm an overdosage (Braverman and Utiger, 1991; Ferguson, 1984; Greenspan, 1994; Williams et al., 1996; Bruner et al., 1998). The canine TSH assay has also been used to evaluate thyroid status in the cat despite incomplete (35%) cross-reactivity for the assay (Ferguson et al., 2007). Measurement of an undetectable serum TSH concentration is a highly sensitive, but only moderately specific, test in support of the diagnosis the confirmation of hyperthyroidism, but should only be used in conjunction with measurement of total and/or free T_4 concentrations (Wakeling et al., 2011). Following surgery, radioiodine or antithyroid medication, the measurement of a normal TSH concentration with total and/or free T_4 is now recommended to evaluate treatment. There is evidence that transient hypothyroidism, associated with low total and/or free T_4 and elevated TSH, is associated with usually mild and occasionally severe renal insufficiency in cats treated for hyperthyroidism (Riensche et al., 2008; van Hoek and Daminet, 2009; Daminet et al., 2014).

The tripeptide TRH is produced in the paraventricular nucleus of the hypothalamus and transported to the pituitary pars distalis by the hypophyseal portal system in the pituitary stalk. In the pituitary gland, TRH binds to specific receptors on the thyrotrope cell and stimulates TSH secretion (Figure 28.2A). In the dog, as in other species, TRH also stimulates the secretion of prolactin. The hypothalamic hormone somatostatin acts to inhibit TSH secretion and may function as a thyrotropin inhibitory factor (Reichlin, 1986).

Negative Feedback Regulation

The negative feedback effect of thyroid hormones (in the free or unbound form) is the principal mechanism regulating TSH secretion. Tonic stimulation by TRH has a permissive role in TSH secretion. The pituitary thyrotope cell completely deiodinates T_4 (derived from the plasma) to T_3 that subsequently inhibits TSH synthesis and secretion through alteration of nuclear receptor binding, mRNA transcription, and protein synthesis. The Type II 5′-deiodinase (D2) mediates the intrapituitary conversion of T_4 to T_3, but, at the same time, T_4 inhibits D2 activity at a posttranslational level by shortening the enzymes cellular half-life through ubiquitination and subsequent proteasomal degradation (Figure 28.2B). D2 and TSH are coexpressed in thyrotrophs and hypothyroidism increases D2 expression in the thyrotroph. As a result, free T_4 (FT_4) concentration and D2-mediated T_3 production correlate negatively with expression of mRNA for TSH (Christoffolete et al., 2006).

Circulating T_4 taken up by the pituitary is the preferred source of T_3 in the pituitary, at least in the rat (Larsen et al., 1981). In human patients with hypothyroidism, thyroid replacement therapy with L-T_4 normalizes serum TSH concentrations only when the serum T_4 value is high normal to slightly high; serum T_3 concentration usually remains within normal range in these patients (Fish et al., 1987; Larsen et al., 1981).

There is also evidence that thyroid hormones may have a direct negative-feedback effect on the hypothalamus to inhibit the release of TRH (Figure 28.2A). Also, TSH and TRH may have “short-loop” and “ultrashort-loop” negative-feedback effects, respectively, upon the hypothalamus to inhibit TRH release. Although pulses of TSH secretion and an evening rise in serum TSH have been described in humans (possibly resulting from a fall in circadian circulating cortisol concentrations), studies in the dog and cat have failed to demonstrate such a circadian rhythm in circulating thyroid hormone concentrations (Fish et al., 1987; Larsen et al., 1981; Magner, 1990; Reichlin, 1986; Bruner et al., 1998; Chiamolera and

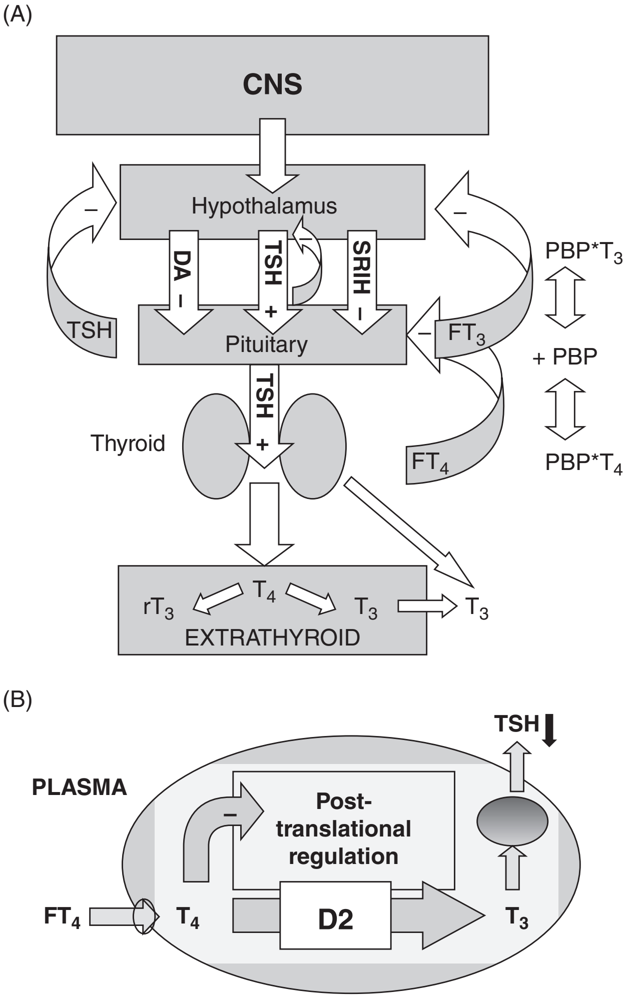


Figure 28.2 **(A)** Hypothalamic–pituitary–thyroid–extrathyroid axis. Please see text for details. TSH, thyrotropin; CNS, central nervous system; DA, dopamine; TRH, thyrotropin releasing hormone; SRIH, somatostatin; TSH, thyrotropin; FT$_3$, free T$_3$; FT$_4$, free T$_4$; PBP, plasma binding proteins; +, stimulation; –, inhibition. **(B)** Postranslational regulation of the D2 enzyme by T$_4$ in the thyrotroph cell. Free T$_4$ (FT$_4$) in the circulation is available to enter the thyrotroph, and the quantity of T$_4$ in the pituitary cell directly and rapidly inhibits the D2 enzyme, which converts T$_4$ to T$_3$. Posttranslational modification of the D2 enzyme by ubiquitination shortens the half-life of the enzyme and ultimately reduces the amount of T$_3$ produced from T$_4$. Most of the T$_3$ binding to the nuclear receptor in the thyrotroph is derived from T$_4$ deiodination. Occupancy of the TR results in down-regulation TSH mRNA and TSH secretion completing the negative feedback loop between FT$_4$ and TSH.

Wondisford, 2009). Pulsatile release of TSH has been confirmed in hypothyroid but not euthyroid dogs, but there was no overlap in values. However, TSH may not be elevated in some confirmed canine hypothyroidism cases, and TSH has been shown to rise initially and eventually fall again in chronic experimentally induced hypothyroid dogs, suggesting either pituitary resistance to lack of negative feedback by thyroid hormones or reduced synthesis and secretion of TSH over time (Kooistra et al., 2000; Diaz-Espineira et al., 2008, 2009).

Metabolism of Thyroid Hormone

The metabolically active thyroid hormones are the iodothyronines L-thyroxine (L-T$_4$) and 3,5,3′- L-triiodothyronine (L-T$_3$) (Figure 28.3). Thyroxine is the main secretory product of the normal thyroid gland. However, T$_3$, which is about three to ten times more potent than T$_4$, as well as smaller amounts of 3,3′,5′-L-triiodothyronine (reverse T$_3$), a thyromimetically inactive product, and other deiodinated metabolites, are also secreted by the thyroid gland of most mammals (Figures 28.2A and 28.3) (Belshaw et al., 1974; Ferguson, 1984; Inada et al., 1975; Kaptein et al., 1993, 1994; Laurberg, 1980).

Although all T$_4$ is secreted by the thyroid, a considerable amount (40 to 60% in the dog) of T$_3$ is derived from extrathyroidal enzymatic 5′-deiodination of T$_4$. Therefore, although it also has intrinsic metabolic activity, T$_4$ has been called a “prohormone,” and its “activation” to the more potent T$_3$ is a step regulated individually by peripheral tissues (Figures 28.3 and 28.4). The vast majority (approximately 90%) of reverse T$_3$ (rT$_3$) is derived from extrathyroidal sources in the dog (Belshaw et al., 1974; Kaptein et al., 1993, 1994; Larsen et al., 1981).

Types and Regulation of Deiodinase Enzymes

The identification of three distinct types of deiodinase enzymes has underscored the importance 1981 of the regulation of T$_3$ production in individual tissues from T$_4$. Type I 5′-D (D1) is found in most tissues, but has its highest activity in liver, kidney, muscle, and thyroid gland. This enzyme is now known to be a selenoenzyme, requiring trace quantities of selenium for optimal activity. Muscle, although it has low enzyme activity, may produce a significant amount (approximately 60% in the rat) of the body’s T$_3$ solely because of its large mass. The physiological role of D1 is the provision of *circulating* T$_3$ during conditions of euthyroidism. This enzyme is capable of “outer-ring” and “inner-ring” deiodination (ring with amino acid moiety) and can deiodinate T$_4$ with a high capacity and is sensitive to the thionamide antithyroid agent, propylthiouracil (PTU). Type II 5′-D (D2) is found in the CNS, pituitary, brown fat, and placenta, and its physiological role is provision of *intracellular* T$_3$. Its role in contributing circulating T$_3$ may increase with primary hypothyroidism. This enzyme acts upon T$_4$ and other compounds with outer-ring iodine at concentrations within the physiological range and is resistant to PTU. Type III deiodinase (D3), which is a 5-deiodinase, removes only inner-ring iodines. D3 is present in placenta, CNS, skin, and fetal brain and liver and has as its physiological role *inactivation* of T$_4$

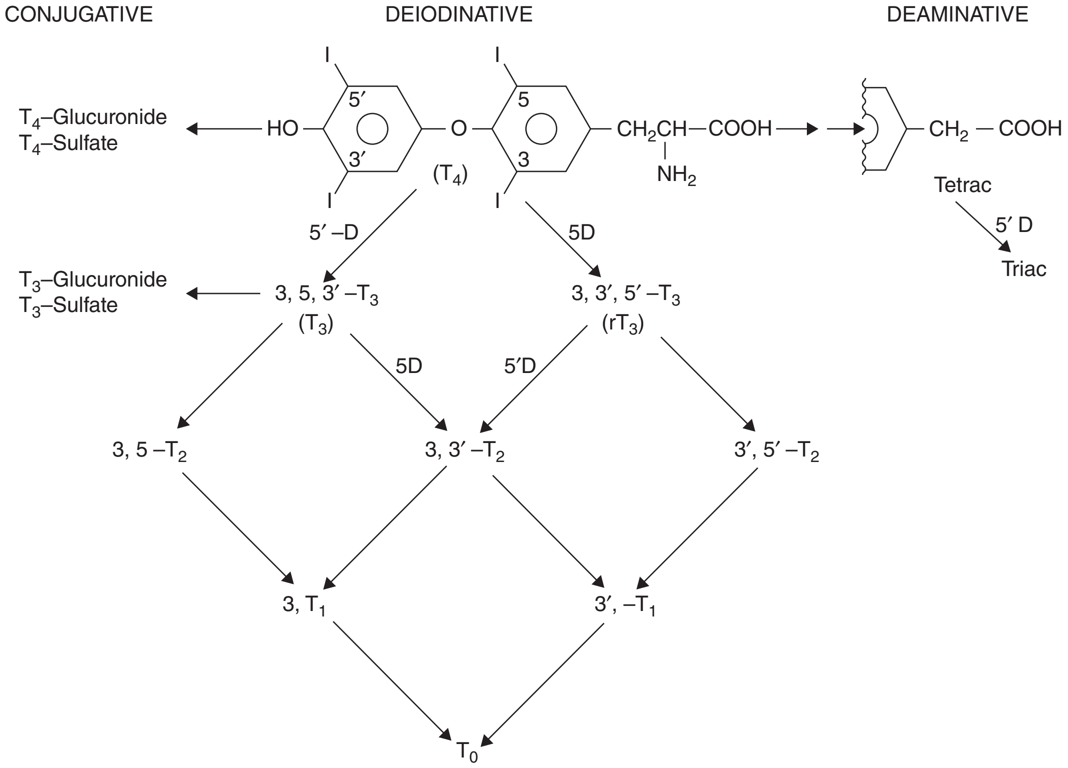


Figure 28.3 Pathways of metabolism of thyroid hormones. 5′-D, 5′-deiodinase; 5-D, 5-deiodinase. Source: Ferguson, 1984. Reproduced with permission of Elsevier.

and T_3 through inner-ring deiodination, with preference for T_3 as a substrate. Relevant to understanding the rationale for L-thyroxine therapy in hypothyroidism, hypothyroidism dramatically reduces the activity of D1 and D3 while increasing the activity of D2. Through this type of regulation, the brain may continue to obtain adequate cellular T_3 levels necessary to prevent or delay neurological dysfunction resulting from T_4 deficiency, while the liver reduces its production of T_3, thereby leading to decreased systemic metabolism (Figure 28.4). It is interesting to note that, in rodents, D1 and D3 are increased in hyperthyroidism resulting in both increased production and degradation rates for T_3. The characteristics of D1 and D2 have been examined in dogs, cats, and cattle. In cats, renal and hepatic D1 has been shown to metabolize T_4 with similar V_{max} and

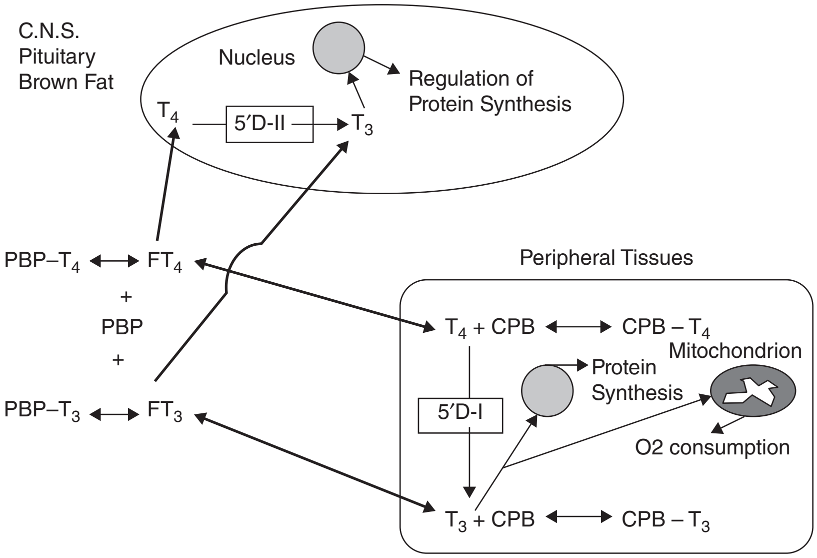


Figure 28.4 Peripheral action of thyroid hormones. T_4 and T_3, in amounts proportional to their free forms (FT_4 and FT_3) in plasma at equilibrium with plasma binding proteins (PBP), are taken up by peripheral tissues such as liver and kidney, which have the type I 5′-deiodinase enzyme (5′-D-I). T_3 from the plasma (or that derived from T_4) interacts with mitochondrial receptors to rapidly increase oxygen consumption and with nuclear receptors to initiate protein synthesis. Cytosolic binding proteins (CBP) buffer the effects of intracellular hormones and provide a relatively unsaturable hormone reservoir. In the brain, pituitary, and brown fat, another isoenzyme of the 5′-deiodinase enzyme (5′-D-II) converts T_4 to T_3. This enzyme is regulated very differently from the type I enzyme. Source: Peterson and Ferguson, 1990. Reproduced with permission of Elsevier.

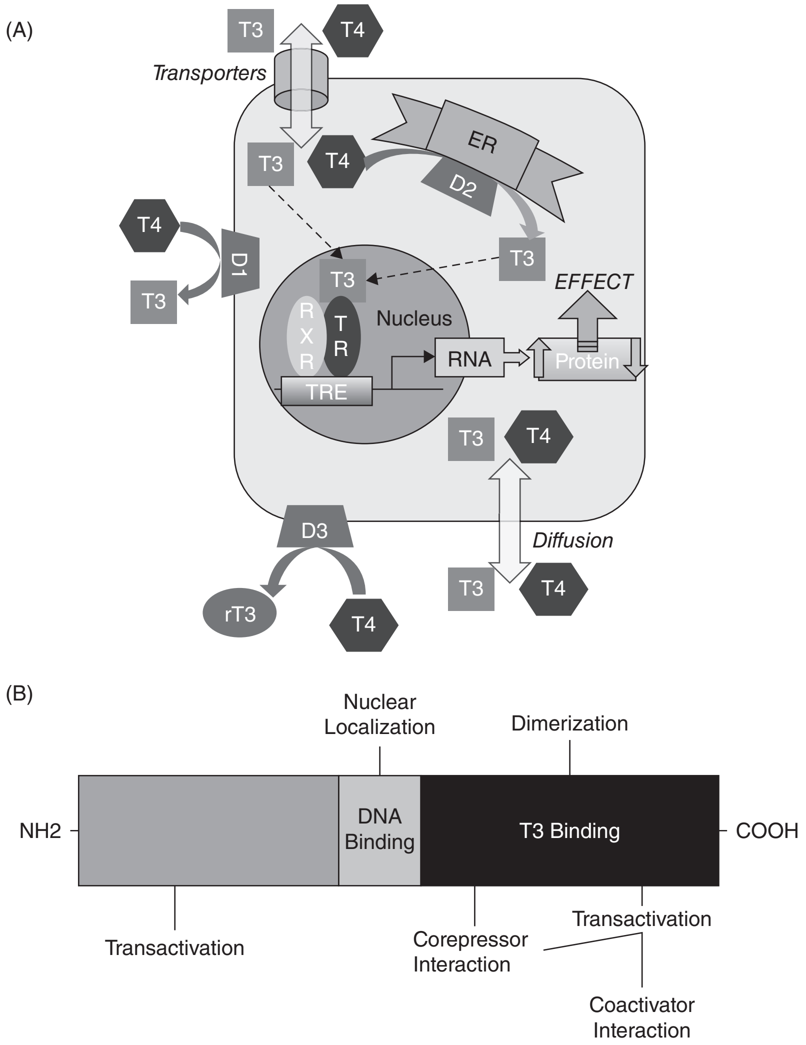


Figure 28.5 **(A)** Cellular localization of thyroid hormone transporters, deiodinases, and nuclear receptors. The D1 and D3 enzymes are plasma membrane-associated enzymes and D1 provides *circulating plasma T_3* to plasma and D3, or 5-deiodinase, leads to inactivation of T_4 to rT_3 or T_3 to 3,3′-T_2. The D2 enzyme is associated with the endoplasmic reticulum in tissues where it dominates (brain, pituitary, brown fat, etc.). Under euthyroid conditions, it provides *intracellular T_3*, but can provide a larger portion of circulating T_3 under hypothyroid conditions when D2 activity is increased. Important plasma membrane transporters for T_4 and T_3, depending upon the tissue, include monocarboxylic acid transporter 8 (MCT8) and organic acid transporter p1c1 (OATp1c1). The lipophilic hormones are also capable of diffusing through cell membranes after dissociating from thyroid binding proteins in the circulation. Once in the cytosol they also can associate with cytosolic thyroid binding protein (CTPB) until delivered to sites of action or metabolism. T_3 enters the nucleus and has high affinity for the thyroid hormone receptor (TR). TR is a heterodimer with the retinoic X receptor (RXR), which binds to the thyroid-response element (TRE), leading to a change in mRNA transcription (increase or decrease) and effects secondary to altered protein synthesis. **(B)** Functional domains of a nuclear thyroid hormone receptor. The functional domains of the thyroid hormone receptor are similar among the four active isoforms (α1, β1, β2, and β3), with the only difference being in the amino-terminal section. Coactivators and corepressors generally interact with the T_3 binding domain, which also dimerizes with the retinoic acid receptor (RXR).

Km of the rat enzyme, but the ability of the enzyme to degrade rT_3 was only about 0.2% of that of rat D1 with a Km 500-fold higher. The D1 enzyme was not identified in the cat's thyroid gland, unlike in rat, dog, and humans. These differences would imply that feline D1 does not metabolize rT_3 under physiological conditions. However, muscle D1 mRNA is stimulated by T_3 treatment of lean cats, as seen in most species. D2 has been identified in human keratinocytes as well as in dog skin. Previous studies have indicated important differences in catalytic specificity between dog and human D1, in particular with respect to the 5′-deiodination of rT_3. While D1 and D2 are found in the microsomal fraction of cells, D3 seems to be a plasma membrane protein, positioning it well to inactivate both excess T_4 and T_3 in tissues such as the placenta and brain (Figure 28.5A) (Baqui et al., 2003; Burrow et al., 1989; Ferguson, 1988; Larsen et al., 1981; Peterson and Ferguson, 1990; Foster et al., 2000; Bianco, 2002; Hoenig et al., 2008).

The process of deiodination continues until the thyroid hormone nucleus is stripped of its remaining iodine molecules thereby allowing iodine to recycle for hormone resynthesis (Figure 28.3). These further deiodinated metabolic products (other than T_3 and T_4) do not have thyromimetic activity. Evidence is accumulating that reverse T_3 may have an important role in regulating thyroid T_4 and T_3's activity in the central nervous system and 3,5-diiodothyronine (3-5-T_2) and 3-monoiodothyronine (3-T_1) may have cardiovascular effects (Ianculescu and Scanlan, 2010; Axelband et al., 2011; Dentice et al., 2013; Orozco et al., 2014). A number of nonthyroidal illnesses and drugs may affect the local tissue regulation of thyroid hormone deiodination. Other pathways of thyroid hormone metabolism include conjugation to form soluble glucuronides and sulfates for biliary or urinary excretion as well as cleavage of the ether linkage of the iodothyronine molecule (Braverman and Utiger, 1991; Burrow et al., 1989; Ferguson, 1984, 1988; Kaptein et al., 1994).

With oral administration of thyroid hormone preparations, the first-pass effect must be considered, as a large quantity of hormone can be conjugated and secreted into the bile where the hormone may either be deconjugated and reabsorbed by bacteria in the large intestine, or eliminated in the feces. The intestinal pool of thyroid hormone is known to be very large. In the dog, over 50% of the T_4 and about 30% of the T_3 produced each day are lost into the feces. In both the dog and cat, the extrathyroidal body stores of T_4 are eliminated and replaced in about 1 day, whereas stores of T_3 are lost and replaced twice daily (Kaptein et al., 1993, 1994). Such fecal wastage is responsible, in part, for the higher daily replacement doses of thyroid hormone required on a per body weight basis in dogs and cats. Despite occasional reports of massive overdoses of levothyroxine products (Hansen et al., 1992), the fecal loss pathway also accounts for the low morbidity with accidental toxicoses with thyroid hormone products in dogs. Nonetheless, demonstrating interindividual differences in sensitivity, it has been reported that a dog became thyrotoxic after coprophagia of feces from another dog in the household receiving a high dose (0.8 mg) of levothyroxine twice daily (Shadwick et al., 2013).

Plasma Hormone Binding of Thyroid Hormone

Thyroid hormones are water-insoluble lipophilic compounds. Their ability to circulate in plasma is dependent upon binding by specific binding proteins, thyroxine-binding protein (TBG) and transthyretin (TTR) or thyroxine-binding prealbumin (TBPA), as well as by albumin itself. Thyroid hormone–binding proteins provide a hormone reservoir in the plasma and "buffer" hormone delivery into tissue (Figure 28.4). TTR and possibly albumin also may serve as intermediary carriers for specific tissue uptake of the hormone by tissues (Mendel, 1989; Pardridge, 1981). The dog has a high-affinity thyroid hormone–binding protein, comparable to TBG in humans, but plasma concentrations of TBG in the dog are only 25% of those in humans. In addition to TBG, TBPA, and albumin, circulating T_4 in canine plasma appears to bind to certain plasma lipoproteins. These include a high-density lipoprotein (HDL_2), which migrates in the alpha$_1$ region on the electrophoretic pattern and a very low-density lipoprotein (VLDL), which migrates in the beta region. At normal serum T_4 concentrations in the dog, about 60% of T_4 is bound to TBG, 17% to TBPA, 12% to albumin, and 11% to the HDL_2. Thyroxine-binding globulin in the dog is not saturated until the total T_4 concentration is six times the normal serum T_4 values, while the other serum proteins are virtually unsaturable (Inada et al., 1975). The cat does not appear to have a high-affinity thyroid-binding protein (such as TBG) but has only TTR and albumin as serum thyroid hormone–binding proteins. Partly as a result of weaker serum protein binding, total T_4 concentrations are lower, the unbound or free fraction of circulating T_4 is higher, and hormone metabolism is more rapid in most domestic animals than in humans (Bigler, 1976; Kaptein et al., 1994; Larsson, 1985; Larsson et al., 1985).

Tissue Thyroid Hormone Uptake: the "Free Hormone" Hypothesis

The free hormone hypothesis, proposed by Robbins and Rall six decades ago (1960) and restated by Mendel (1989), states that it is the unbound fraction of hormone that is available to tissues and therefore proportional to the action, metabolism, and elimination of that hormone. This hypothesis has stood the clinical test of time; direct or indirect measurements of free T_4 have been a mainstay in the diagnosis of thyroid disease in human and veterinary medicine. There is also strong evidence that certain cell types actively transport or exchange thyroid hormone from the plasma into the cytosol. The presence of a plasma membrane protein specific for thyroid hormone transport (see Section Plasma Membrane Transporters and Cytosolic Binding Proteins) certainly reflects the premium the cell is willing to pay to facilitate entry and possibly concentrate thyroid hormone. However, the serum binding proteins, particularly albumin and TTR, also serve the role of distributing the hormones to specific tissues. TTR carries both thyroid hormones and retinoic acid in the circulation. Most theories of thyroid hormone exchange have assigned a passive "reservoir" role to cytosolic thyroid hormone-binding proteins (CTBP; CBP in Figure 28.4), the proteins that retain thyroid hormone in a predominantly bound state inside the cell. Little is known about the regulation of these proteins, which are often called "intracellular albumin" for their low specificity, low affinity, and high capacity. However, in renal cytosol, the affinity of CTBP may be acutely regulated by cellular redox potential, increasing when NADPH levels are high. CTBP has been shown to be identical to the protein μ-crystallin (Burrow et al., 1989; Hashizume et al., 1987; Kaptein et al., 1994; Mendel, 1989; Pardridge, 1981; Suzuki et al., 2007).

Irrespective of the mechanisms, the following observations in the clinical patient must be recognized:

1) The linear correlation between the serum free T_4 concentration, rate of hormonal degradation, and basal metabolic rate in humans.
2) The inverse correlation between the serum free T_4 concentration and the cellular distribution volume of T_4, which exists in all subjects regardless of thyroid state.
3) The positive correlation between the in vitro perfused organ free T_4 concentration and tissue T_4 uptake and T_3 production in vivo and in vitro. Most researchers agree that it is the steady-state tissue concentrations of hormone that are the driving force for thyroid hormone metabolism and action (Mendel, 1989).

In the healthy euthyroid dog or cat, about 0.1% of total concentration of serum T_4 is free (i.e., not bound to thyroid hormone-binding proteins), whereas about 1% of circulating T_3 is free (Ferguson and Peterson, 1992; Kaptein et al., 1994). The proportion of free hormone may change in response to drug administration or illness. For example, in obese cats, increased nonesterified free fatty acids compete for hormone binding, resulting in an increase in free serum thyroid hormone concentrations, and may have a similar effect to compete with cellular binding, leading to a form of thyroid hormone resistance to negative feedback manifested by an increase in total and/or free T_4 concentrations. However, it appears that thyroid status of the animal does not change, since the absolute level of the free hormone concentrations tends to soon return to within normal range or remains relatively constant (Ferguson, 1988, 1989b, 1994; Ferguson et al., 2007).

Most evidence suggests that the thyroid hormone uptake by tissues is proportional to, but not limited to, the free or unbound fraction of circulating hormone. Approximately 50 to 60% of the body's T_4 and 90 to 95% of the body's T_3 is located in the intracellular compartment (Fish et al., 1987). Certain organs, particularly the liver and kidney, can concentrate thyroid hormones and exchange hormone rapidly with the plasma. In humans, about 60% of the intracellular T_4 is in these *rapidly equilibrating* tissues (liver and kidney), whereas only 6% of the intracellular T_3 is in these tissues. About 80% of all extrathyroidal T_3 is located in the *slowly equilibrating* tissues (e.g., muscle, skin), while only 20% of intracellular T_4 is in this compartment. As a result, most of the body's T_4 is located in plasma, interstitial fluid, liver, and kidney. The majority of the body's extrathyroidal T_3 is in the cells of the muscle and skin and in a conjugated form in the intestinal tract. Kaptein et al. (1994) have used a three-pool model to describe the distribution and metabolism of thyroid hormone metabolites and iodide (Figure 28.6). Table 28.1 compares the model parameters for T_4, T_3, rT_3, and iodide in dogs, cats, and humans.

As a principle impacting the diagnosis and treatment of thyroidal diseases, the tissue-specific regulation of deiodinases and TR receptor subtypes (see Section Correlation of Clinical Effects with Cellular Actions) suggests that the determination of thyroid status really must be established on a tissue-by-tissue basis.

Metabolic Clearance Rates

The plasma half-life of T_4 in the dog has been estimated to be 8 hours (Kaptein et al., 1993, 1994), 10–16 hours (Fox and Nachreiner, 1981), and 11.6 hours (Le Traon, 2008), and 11 hours in the cat (Kaptein et al., 1994), compared to a plasma half-life of about 7 days in humans. Similarly, the plasma half-life of T_3 in the dog has been estimated to be 5–6 hours, compared to 24–36 hours in humans (Table 28.2). Studies in the normal cat indicate that the plasma half-lives of T_4 and T_3 are similar to that of the dog (Fox and Nachreiner, 1981; Kaptein et al., 1994). Despite these rates of plasma clearance, the suppressive effect on pituitary TSH secretion appears to persist at least 24 hours after a dose, suggesting that intrapituitary T_3 persists longer than the serum half-life (Ferguson, 2007; Le Traon, 2009). Therefore, as with many lipophilic nuclear receptor–mediated hormones, the plasma disappearance rates may underestimate the extent or duration of biological action.

Extrathyroidal Factors Altering Thyroid Hormone Metabolism

Effect of Illness and Malnutrition in Humans

In humans, and increasingly recognized in domestic animals, a wide range of clinical conditions, such as chronic starvation or malnutrition, surgery, diabetes mellitus, hepatic and renal disease, and chronic systemic illness, may result in decreased serum T_3 concentrations together with elevated serum rT_3 values (Araujo and

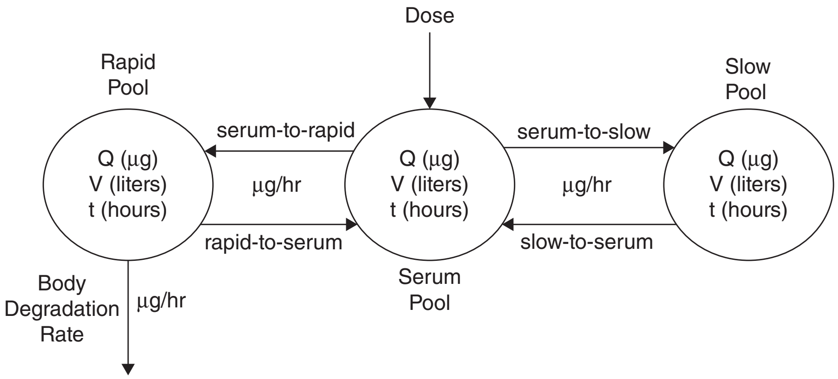


Figure 28.6 Compartmental model of thyroid hormone metabolism.

Table 28.1 Thyroid hormone and iodide kinetics in normal dogs, cats, and humans using a three-pool model. Source: Kaptein et al., 1994. Dog and human data from Kaptein et al., 1993. Cat data from Hays et al., 1988.

	Dogs	Cats	Humans
T_4			
Plasma concentration (ng/dL)	2.8	1.68	6.8
Free fraction (%)	0.102	0.056	0.030
Total mean residence time (d)	0.54	0.69	6.8
Clearance rate (L/[kg·d])	0.24	0.38	0.017
Degradation rate (μg/[kg·d])	6.81	6.2	1.14
Total pool size (μg/kg)	3.7	4.3	7.8
Distribution (%)			
Plasma pool	46	13	32
Rapidly equilibrating pool	23	19	26
Slowly equilibrating pool	31	68	42
T_3			
Plasma concentration (ng/dL)	43	32	140
Free fraction (%)	1.426	0.48	0.299
Total mean residence time (d)	0.40	0.62	1.50
Clearance rate (L/[kg·d])	1.99	1.77	0.32
Degradation rate (μg/[kg·d])	0.84–0.89	0.52–2.2	0.43–0.46
Total pool size (μg/kg)	0.34	0.30	0.66
Distribution (%)			
Plasma pool	7	3.6	9
Rapidly equilibrating pool	29	18	19
Slowly equilibrating pool	64	79	73
Reverse T_3			
Plasma concentration (ng/dL)	22	—	13
Free fraction (%)	0.560	—	0.111
Total mean residence time (d)	0.12	—	0.16
Clearance rate (L/[kg·d])	1.44	—	1.78
Degradation rate (μg/[kg·d])	0.32–0.42	—	0.23–0.33
Total pool size (μg/kg)	0.039	—	0.036
Distribution (%)			
Plasma pool	31	—	15
Rapidly equilibrating pool	29	—	29
Slowly equilibrating pool	40	—	55
Inorganic iodide[a]			
Plasma mass (μg/kg)[b]	21	—	1.67
Total mean residence time (d)	1.07	0.82	0.35–0.36[c]
Clearance rate (L/[kg·d])	0.43	0.63	1.00–1.01
Total distribution volume (L/kg)	0.46	0.44	0.35–0.36
Distribution (%)			
Plasma pool	19	11	13[c]
Rapidly equilibrating pool	9	32	38
Slowly equilibrating pool	72	57	50

Parameter values were estimated using a three-pool model. Only mean values are presented. Conversion to SI units: Total T_4 in μg/dL × 12.87 = nmol/L; total T_3 and reverse T_3 in ng/dL × 0.01536 = nmol/L.

[a] Thyroid gland unblocked for all species.

[b] Dog data from Belshaw et al., 1974.

[c] Data from Hays and Solomon, 1965 (reanalyzed in three-pool model); Belshaw et al., 1974.

Carvalho, 2011). This "low T_3" syndrome results from inhibition of 5′-deiodinase, the enzyme necessary for conversion of T_4 to T_3 and the conversion of rT_3 to 3,3′-T_2 (Braverman and Utiger, 1991; Burrow et al., 1989; Kaptein et al., 1994; Kaptein, 1986; Daminet and Ferguson, 2003).

The reduction in the production of T_3, the most potent thyroid hormone, appears to be a beneficial adaptive mechanism by which the body serves to limit the loss of protein and perhaps blunt the metabolic rate during illness. Maintenance of serum T_3 concentrations (with triiodothyronine replacement therapy) in euthyroid fasting humans leads to excessive nitrogen excretion and blunting of the pituitary's TSH response to TRH, as if there was a state of hyperthyroidism. The body of evidence does not support the contention that lowering of serum T_3 during malnutrition and illness is associated with "tissue" hypothyroidism. Also, outside of these

Table 28.2 Thyroxine kinetics in dogs, cats, and humans following intravenous, subcutaneous, and oral L-thyroxine administration. Source: Kaptein et al., 1994. Data on dogs and humans from Kaptein et al., 1993.

	Dogs	Cats	Humans
T_4 degradation rate (μg/[kg·day])	6.81	6.2	1.14
Intravenous			
Time to peak (hour)	0.02–0.03	0.02–0.03$_a$	0.02–0.03
Total mean residence time (hour)	13.0	16.6^a	164
Terminal half-life (h)	7.6	10.7^b	168
Subcutaneous			
Dosage (μg/[kg·day])	5–7	—	—
Absorption (%)	100^c	—	81
Time to peak (h)	1.5–1.8	—	72
Terminal half-life (h)	14.7	—	120
Oral			
Dosage (μg/[kg·day])	20–40^c	20–30^c	2.11
Absorption (%)	10–50^c	10.5^c	50–80
Time to peak (h)	—	3–4^c	2–4
Terminal half-life (h)	7.6	10.7^c	168

Conversion to SI units: Total T_4 in μg × 1.287 = nmol.

[a] Data from Hays et al., 1988.

[b] Data from Hays et al., 1992.

[c] Data from Hulter et al., 1984.

regulatory mechanisms, there is, as yet, no evidence that a congenital or acquired 5′-deiodinase deficiency exists in animals or in specific tissues (Braverman and Utiger, 1991; Ferguson, 1984, 1988; Kaptein et al., 1993, 1994; Kaptein, 1986).

In acute and severe illnesses in humans, serum T_4 and T_3 concentrations may also fall, in what is called the "low T_4 state of medical illness." Impaired serum protein binding of T_4 caused by inhibitors of binding (such as free fatty acids) or a reduction in binding protein concentration results in reduced total serum T_4 concentrations and increased free fractions of T_4. In most cases, however, the absolute free T_4 concentrations remain normal. A fall in serum TSH concentrations may also contribute to the subnormal serum T_4 concentrations, especially in human patients treated with dopamine or glucocorticoids, drugs inhibiting TSH release. No studies have examined systematically the benefit or detriment of thyroid hormone therapy in domestic animals; however, studies of critically ill patients with low serum T_4 and T_3 concentrations have revealed that thyroxine therapy is not beneficial and fails to improve survival (Kaptein, 1986; Kaptein et al., 1993, 1994).

The effects of nonthyroidal illness on thyroid hormone metabolism in the dog are less well characterized than in humans. In the dog, depressed serum T_4 concentrations have been reported in various nonthyroidal illnesses such as hyperadrenocorticism (e.g., Cushing's syndrome), diabetes mellitus, hypoadrenocorticism (e.g., Addison's disease), chronic renal failure, hepatic disease, as well as a variety of other critical medical illnesses requiring intensive care (Ferguson, 1984, 1988, 1994, 2007; Ferguson and Peterson, 1992).

Effect of Drugs on Thyroid Function

This topic was reviewed by Daminet and Ferguson in 2003. A variety of drugs may impair plasma or tissue binding of the thyroid hormones or alter thyroid hormone metabolism. Drugs used in veterinary medicine that are most likely to alter circulating thyroid hormone concentrations include the glucocorticoids, anticonvulsants, quinidine, salicylates, phenylbutazone, and radiocontrast agents. The mechanisms by which these drugs exert their effect vary. Quinidine and other membrane-stabilizing drugs may inhibit 5′-deiodinase. Salicylates, furosemide, and oleic acid may directly displace thyroid hormone from plasma binding sites (Daminet and Ferguson, 2003; Ferguson, 2007; Ferguson et al., 2007). Phenylbutazone appears also to have a direct antithyroid (goitrogenic) effect in some species, having been shown to decrease total and free T_4 in the horse (Ramirez et al., 1997). In vitro, it appears to decrease serum hormone binding. Radiocontrast agents (e.g., diatrizoate, iopanoic acid, ipodate, tyropanoate, and metrizamide) may act by preventing the uptake of T_4 by tissue, by directly inhibiting 5′-deiodinase, or by releasing the iodine they contain to exert an antithyroid effect on the thyroid gland. No studies have been reported in domestic animals to evaluate the influence of these iodine-containing drugs on thyroid function tests or on subsequent radioiodine uptake (Ferguson, 1984, 1989b, 1994).

Exogenous glucocorticoids have also been shown to have a profound effect on thyroid function tests in the dog, but similar studies have had little effect on serum T_4 levels in the cat. A single high immunosuppressive dosage of glucocorticoid (2.2 mg/kg prednisone, 0.6 mg/kg dexamethasone) will lower serum T_3 but not serum T_4 concentrations in the dog (Kemppainen et al., 1983; Laurberg and Boye, 1984). Serum T_3 concentrations may be decreased because of glucocorticoid inhibition of 5′-deiodinase or simply because of a reduced availability of plasma T_4, the substrate for the enzyme. Most dogs on chronic, high-dose, daily glucocorticoid therapy will have very low or undetectable serum T_4 concentrations, as well as subnormal serum T_3 values (Ferguson and Peterson, 1992; Kaptein et al., 1992; Kemppainen et al., 1983; Moore et al., 1993; Torres et al., 1991). Based upon electron microscopic examination of thyroid tissue, it was postulated that glucocorticoids may interfere with thyroid hormone secretion by inhibiting lysosomal hydrolysis of colloid in the follicular cell (Woltz et al., 1983). *Immunosuppressive* dosages (1.1–2 mg/kg q 12 h) of prednisolone in dogs for 3 to 4 weeks significantly decreased serum total T_4 and to a lesser extent, FT_4 concentrations. These changes were observed as soon as 1 day after initiation of treatment (Torres et al., 1991; Daminet et al., 1999). Endogenous TSH was not affected by 3 weeks of an immunosuppressive dosage of prednisone, but the assay may not allow detection of a mild decrease. No changes in serum total T_4 levels were seen after a month of prednisone administration at an *antiinflammatory* dosage (0.5 mg/kg q 12 h) (Moore et al., 1993). However, a decrease in total T_3 concentrations with a supranormal T_4 increase after TSH administration was observed. Another study also showed that prednisone at an antiinflammatory dosage of 1 mg/kg/day had no effect on TSH concentration of dogs with experimental hypothyroidism, but higher dosages have not been studied. A subsequent study in T_4-supplemented hypothyroid dogs showed that prednisone at a dosage of 1 mg/kg PO q 24 h for 7 days decreased total T_4, while FT_4 was unchanged, suggesting that FT_4 may be less affected by daily prednisone administration. Subsequent antiinflammatory doses of prednisone administered every other day did not alter T_4 or TSH concentrations (O'Neill et al., 2010). In summary, glucocorticoids can markedly decrease total T_3 and T_4 concentrations and to a lesser extent FT_4 concentrations in dogs. Therefore, thyroid function test results should be interpreted carefully in any dog receiving glucocorticoids if prescribed an immunosuppressive dosage or for a period greater than 1 month (Ferguson, 1984, 1994; Moore et al., 1993; Daminet and Ferguson, 2003). In the horse, 50 g dexamethasone q 12 h topically for 10 days led to a 30–40% decrease in both T_3 and T_4 with nadir at day 2 for serum T_3 concentration and day 6 for serum T_4 concentration. Neither hormone concentration had recovered by 11 days after discontinuation of therapy (Abraham et al., 2011).

Other drugs have been well documented to alter thyroid hormone metabolism or serum or tissue binding of the thyroid hormones in the dog. The anticonvulsants diphenylhydantoin and phenobarbital, which are mixed function oxidase inducers, consistently decrease serum T_4 concentrations. Phenobarbital increases the rate of clearance of T_4. Increased hepatic deiodination of thyroid hormones, biliary clearance, and fecal excretion result in decreased concentrations of circulating thyroid hormones. The *short-term* administration (3 weeks) of phenobarbital to dogs did not affect total or free T_4 nor TSH serum concentrations in beagle dogs. However, several studies have looked at the *long-term* effects of phenobarbital administration. Total and free T_4 can be decreased to a range consistent with hypothyroidism. Endogenous TSH concentrations can remain within reference range or be slightly increased. Serum phenobarbital concentrations did correlate with the decrease in total and free T_4 concentrations in one study, but not in another. The reduction of free T_4 concentration particularly supports the idea that phenobarbital influences the steady-state clearance of T_4. Difficulty of interpretation of drug effects is compounded by the observation in dogs that seizure activity reduces serum total T_4 concentrations in proportion to the frequency of seizure episodes (von Klopmann et al., 2006). In one study, thyroid function normalized 1 to 4 weeks after discontinuation of phenobarbital. It is therefore recommended, when this is an option clinically, to evaluate thyroid function at least 4–6 weeks after discontinuation of phenobarbital (Kantrowitz et al., 1999; Gaskill et al., 1999; Müller et al., 2000; Gieger et al., 2000; Daminet et al., 1999; McClain et al., 1989; Curran and DeGroot, 1991; Johnson et al., 1993; Barter and Klaassen, 1994; Theodoropoulos and Zolman, 1989; Liu et al., 1995; DeSandro et al., 1991; Attia and Aref, 1991).

The antiepileptic potassium bromide is a halide chemically related to iodide and could potentially interact with iodine in the thyroid gland. In rats, bromide produces a relative iodine deficiency, thereby interfering with iodine uptake, iodine transport, and iodination of tyrosine and tyrosyl residues on thyroglobulin. In one study of epileptic dogs and another of healthy dogs receiving KBr at therapeutic dosages up to 6 months, no abnormal thyroid function test results were seen (Kantrowitz et al., 1999, Paull et al., 2000).

A study evaluated the effect of a standard dosage of trimethoprim/ sulfamethoxazole on thyroid function tests in dogs with pyoderma and normal baseline serum thyroxine concentrations. The average serum T_4, but not T_3, concentration fell significantly during the treatment period of 6 weeks. The TSH response also fell in several dogs and radionuclide imaging suggested that the preparation (likely the sulfa component) interfered with iodine metabolism by the thyroid gland. The thyroid carcinogenic potential of sulfonamides, which has resulted in restriction of some forms in food animals, is likely due to its goitrogenic potential, chronic elevation of serum TSH, and subsequent stimulation of thyroid growth (Hall et al., 1993). Sulfonamides are known goitrogens in domestic species because they can markedly interfere with thyroid hormone synthesis through reversible inhibition of TPO activity and reduction of serum concentrations of thyroid hormones. With reduced negative feedback, there is an increase in pituitary secretion of TSH, which induces proliferative changes in the thyroid gland. Long-term administration of sulfonamides and prolonged stimulation of the thyroid gland by TSH have been associated with thyroid neoplasia in rats. There is considerable interspecies variation with respect to TPO inhibition by sulfonamides. For example, only mild effects of sulfonamides are observed on human thyroid function. Several prospective studies have evaluated the effects of sulfonamide administration on canine thyroid function. Trimethoprim-sulfadiazine administered at a dosage of 15 mg/kg q 12 h to healthy dogs for 4 weeks had no effect on serum total T_4 or T_3 or free T_4 concentrations or upon the results of TSH stimulation tests. When trimethoprim-sulfadimethoxazole was administered to dogs with pyoderma for 6 weeks at a higher dosage of 30 mg/kg q 12 h, serum total T_4 and T_3, and FT_4 concentrations decreased. Half of the dogs in this experiment had total thyroxine (TT_4) levels below the reference range at the end of the treatment, which could easily have resulted in the inappropriate diagnosis of primary thyroid failure. In another study, the same dosage of trimethoprim-sulfadimethoxazole was given to healthy dogs for 6 weeks, and thyroid hormones were markedly decreased and endogenous TSH increased as soon as 7 days after initiation of the treatment. Thyroid imaging showed increased uptake of pertechnetate, and thyroid gland biopsies revealed hyperplasia of thyroid follicles and absence of colloid production. These findings further support the concept that sulfonamides act as goitrogens, resulting in primary hypothyroidism with secondary changes associated with the proliferative effects of TSH. The effects of sulfonamides on thyroid function are species specific, and dosage and duration dependent. Normalization of thyroid function test results in dogs after cessation of sulfonamide administration can take up to 8 or 12 weeks. Sulfa drugs can lead to clinical hypothyroidism and even goiter in some dogs (Cohen et al., 1980, 1981; Lagler et al., 1976; Panciera and Post, 1992; Post et al., 1993; Hall et al., 1993; Campbell et al., 1996; Gookin et al., 1999; Taeymans and O'Marra, 2009).

Many nonsteroidal antiinflammatory drugs (NSAIDs) have been shown to alter thyroid function tests in humans, since circulating thyroid hormones are highly protein bound, and various NSAIDs can displace thyroid hormones from serum protein-binding sites. Short-term administration of therapeutic dosages of salicylates leads to a transient increase in unbound hormone levels and suppression of TSH concentrations. After long-term treatment with salicylates, a new steady-state is reached, reflecting an increased T_4 turnover rate, and serum total T_4 concentrations have been reported to be reduced 20–40%. Free T_4 concentrations can be unchanged or decreased, and TSH concentrations return to the reference range within a few weeks of treatment. However, a 60-day double-blind study of osteoarthritic euthyroid dogs given placebo, meloxicam, carprofen, or chondroitin sulfate/glucosamine identified no drug-associated changes in serum total and free T_4 concentrations or TSH (Sauve et al., 2003).

Mechanisms of Thyroid Hormone Action

Correlation of Clinical Effects with Cellular Actions

Thyroid hormone (T_4 and/or T_3) acts on many different cellular processes via specific ligand–receptor interactions with the nucleus, the mitochondria, and the plasma membrane (Figure 28.4). The effects of thyroid hormone are seen in most tissues throughout the body. Although both L-T_4 and L-T_3 have intrinsic metabolic activity, L-T_3 is three to ten times more potent in binding to the nuclear receptors and similarly more potent in stimulating oxygen consumption (Table 28.3). Except for the deaminated form of T_4 and T_3 (tetraiodothyroacetic acid (Tetrac) and triiodothyroacetic acid (Triac), respectively),x most thyroid hormone metabolites have little thyromimetic activity. Reverse T_3 (3,3′,5′-T_3) has recently been linked to some potential developmental effects in the central nervous system.

The effects of thyroid hormone can generally be divided into those that are rapid and evident within minutes to hours of administration, such as stimulation of amino acid transport and mitochondrial oxygen

Table 28.3 Relative nuclear binding affinity of thyroid hormone analogs to T_3 . Source: Oppenheimer, 1983. Reproduced with permission of Elsevier.

	Relative binding affinity ($T_3 = 1$)	
Analog	In vitro	In vivo
L-T_3	1.0	1.0
D-T_3	0.6	0.7
Triiodothyroacetic acid (triac)	1.6	1.0
Isopropyl T_2	1.0	1.0
L-T_4	0.1	0.1
Tetraiodothyroacetic acid (Tetrac)	0.16	0.05
3,3′,5′-T_3 (reverse T_3)	0.001	0
Monoiodotyrosine	0	0
Diiodotyrosine	0	0

cons1981umption, and those that require protein synthesis and a longer period of time (usually no sooner than 6 hours) to be manifested. Of course, the clinical manifestations may require weeks to months to clearly appreciate. About one-half of the increment in oxygen consumption produced by thyroid hormone has been related to activation of the plasma membrane-bound Na^+,K^+-ATPase, which, at least in the kidney and liver, is secondary to increases in passive K^+ fluxes caused directly and primarily by thyroid hormone via as yet undetermined mechanisms. These changes have been linked directly to the calorigenic effect of thyroid hormone. The rapid hormone effects can be observed clinically in the hypothyroid patient starting on thyroid replacement therapy by signs such as increased physical and mental activity (Braverman and Utiger, 1991; Burrow et al., 1989; Greenspan, 1994).

Chronic effects of thyroid hormone invariably are related to the cellular actions of the hormone requiring interaction with nuclear thyroid hormone receptors (TRs) followed by an increase in protein synthesis (Brent, 2012). Clinically, these are effects such as growth, differentiation, proliferation, and maturation. A common clinical presentation of thyroid insufficiency is bilateral symmetrical alopecia, the result of diminished turnover of shafts of hair within the hair follicle, resulting in greater amounts of telogen hairs. Such changes are slow in onset and, upon treatment, slow to resolve.

Plasma Membrane Transporters and Cytosolic Binding Proteins

The details of the following processes will vary depending upon the specific cell type. Free thyroid hormone (TH), translocated by passive diffusion or specific plasma membrane transporters (monocarboxylate transporter 9 (MCT-8) or organic acid transporter p1c1 (OATp1c1), depending upon the species) (Heuer and Visser, 2009) enters into the cell and binds to cytosolic thyroid binding protein (CTBP, now known as μ-crystallin) to maintain intracellular solubility and buffering, acting as a form of intracellular hormone storage. There is evidence that the affinity of μ-crystallin for TH may be increased by NADPH, resulting in another possible mechanism for an impact on cellular action. Cytosolic TH presumably exchanges with specific nuclear thyroid hormone receptors (Figure 28.5A). MCT-8 inhibitors are in development (Braun et al., 2012). It has been hypothesized that the movement of thyroid hormone from the circulation to the cerebrospinal fluid (CSF) involves entry into the choroid plexus epithelial cells via basolateral membrane TH transporters, where it binds to transthyretin (TTR) synthesized by these cells, and then moves out with TTR into the CSF or via brush border TH membrane transporters (Richardson et al., 2015).

Nuclear Thyroid Hormone Receptors

Most, but not all, of the cellular actions of thyroid hormone can be linked to binding to nuclear receptors. Thyroid hormones act by binding to a specific nuclear thyroid hormone receptor (TR), which is a heterodimer with the retinoid X receptor (RXR). TRs are members of a family of nuclear receptors similar to the v-erb A receptor, which is a receptor for the avian erythroblastosis virus, including the glucocorticoid, mineralocorticoid, estrogen, progestin, vitamin D_3, and retinoic acid receptors. There are three major functional domains of this receptor, one binding DNA, one binding the ligand, and two major transcriptional activation domains (Figure 28.5B). T_3 has a higher binding affinity for TRs than does T_4, leading to its function as the most potent thyroid hormone analogue.

The presence of multiple forms of the thyroid hormone receptor, with tissue and development stage-dependent differences in their expression, leads to an extraordinary level of complexity in the physiological effects of thyroid hormone. There are two TR genes (TRα and TRβ). Alternatively spliced gene products from each of these genes produce active (TRα-1 and TRs β1, β2, and β3) and inactive (α-2 and α-3) gene products, which are expressed in a tissue-specific manner. TRβ-2 is the subtype of the receptor in the cochlea, hypothalamus, and pituitary gland; it mediates negative feedback and is down-regulated by T_3. TRβ-1 is expressed in all tissues, with the highest levels in kidney, liver, brain, and heart. TRα-1 mRNA is expressed most highly in the brain, with lower levels in skeletal muscle, heart, and lungs. TRβ-3 mRNA is not very abundant but is found in highest levels in the liver, kidneys, and lungs. TRα-2 is found only in the heart, and in human, dog, and guinea pig, but not rodents. While not binding T_3, TRα-2 does bind the thyroid-response element (TRE) and may antagonize TRα-1's action. In addition, there are truncated proteins, with unclear physiological roles, which do not bind either T_3 or the TRE (Blange et al., 1997; Larsen et al., 2003; Bernal and Morte, 2013).

For a function directly regulated by a TR, the binding affinity of thyroid analogues directly predicts the biological activity of that analogue (Table 28.3). The binding of T_3 to the TR-TRE complex leads to stimulation or inhibition of the mRNA and protein synthesis (Figure 28.5A,B). Upon binding TH, a conformational change in the TR results in dissociation of repressors of transcription, which are replaced by coactivators, including those stimulating DNA acetylation or histone acetyltransferase activity. The latter enzyme causes dissociation of thyroid hormone–regulated genes, which then bind the transcriptional initiation complex. The action of T_3 is terminated by its dissociation from the receptor or by ubiquitination and proteasomal degradation of the hormone-bound complex.

L-T_4 is the preferred choice of thyroid hormone for therapy of hypothyroidism; although T_3 activates cellular thyroid hormone–dependent genes with highest potency, T_4 negatively regulates its own activation to T_3 by posttranslationally down-regulating D2 in the pituitary and other tissues, reducing TSH, and therefore, indirectly as well as directly, inactivating D1 (Larsen et al., 2003). Topological studies suggest that D1 is a plasma membrane protein with its catalytic site on the cytosolic surface, therefore facilitating access of the enzyme to circulating rT_3 and T_4 as well as the entry of produced T_3 into the plasma. D2 is localized to endoplasmic reticulum. These subcellular distribution patterns of the 5′-deiodinases may explain why T_3 produced by D2 is most likely to impact nuclear receptor occupancy and T_3 produced by D1 is most likely to be released to the circulation (Bianco et al., 2002; Larsen et al., 2003; Bianco and McAninch, 2013) (Figure 28.5A).

Extranuclear Actions of Thyroid Hormone

Some of the actions of thyroid hormone occur in the absence of new protein synthesis. Nongenomic actions, which include stimulation of cardiac plasma membrane Na^+ channel, the inward-rectifying and voltage-activated K^+ channels, Na^+/H^+ exchange, and the calcium pump (Ca^{2+}-ATPase), have been shown in intact cells and isolated plasma membranes. Actions on channels or pumps may contribute to setting of basal activity of these transport functions, perhaps by influencing protein kinases that modulate channel activity. Some of these nongenomic effects are now attributed to binding to αvβ3 integrin plasma membrane receptors. By interaction with these receptors, T_4 or T_3 can activate ERK1 or ERK2, which then serine phosphorylate TRb1, ERa, STAT1a, and p53 nuclear receptors that lead to mRNA synthesis for proliferative and proangiogenic proteins like basic fibroblast growth factor (bFGF) and epidermal growth factor (EGF). The integrin receptor also cross-talks with the FGF and EGF receptors. The thyroid hormone deamination product tetraiodothyroacetic acid (Tetrac) has been shown to block both the integrin and FGF receptors, and has therefore been explored as an anticancer agent (Davis et al., 2010).

A mitochondrial T_3 receptor has also been identified and has been postulated to mediate the activity of the mitochondrial adenine nucleotide translocase (ANT), indirectly stimulating oxygen consumption. The density of β-adrenergic receptors on cardiomyocytes is increased by T_3 within 2 hours even when protein synthesis is inhibited. Another nongenomic effect already described is the posttranslational inhibition by ubiquitination of the Type II 5'-deiodinase (D2) (Greenspan, 1994; Davis and Davis, 2002; Senese et al., 2014).

Physiological and Pharmacological Effects of Thyroid Hormone

Thyroid hormones, in physiological quantities, are anabolic. Working in conjunction with growth hormone and insulin, protein synthesis is stimulated and nitrogen excretion is reduced. However, in excess (i.e., hyperthyroidism), they can be catabolic, with an increase in gluconeogenesis, protein breakdown, and nitrogen wasting. Table 28.4 summarizes the multiple organ effects of thyroid hormone and the clinical manifestations of hormone deficit (hypothyroidism).

Calorigenesis and Thermoregulation

Thyroid hormones increase oxygen consumption and heat production to a large extent by stimulating uncoupling proteins (UCPs) and ANT in the mitochondria. UCPs (in particular UCP1 and UCP2) induce a H^+ leak to create inefficiency in ATP utilization resulting in heat generation (Silva, 2011). Thyroid hormone also stimulates Na^+,K^+-ATPase in all tissues except the brain, spleen, and testis. As such, thyroid hormones determine the basal (resting) metabolic rate of the animal. This results in the development of mental dullness, lethargy, and an unwillingness to exercise. The reduced basal metabolic rate results in hypothermia and the tendency for the animal to seek heat (Greco et al., 1998). Once-daily administration of 0.022 mg/kg was sufficient to normalize basal metabolic rate in dogs when measured by indirect calorimetry. Clinical signs improved in 93% of dogs with either improvement or complete resolution. This dosage was also shown to suppress TSH values to undetectable levels in most thyroidectomized dogs (Ferguson and Hoenig, 1997).

Effects on Growth and Maturation

The fetus obtains thyroid hormone from maternal sources during the first trimester of gestation in most mammals, after which the fetal thyroid function matures. Thyroid hormones are crucial for growth and development of the skeleton and central nervous system. Therefore, in addition to the well-recognized signs of adult-onset hypothyroidism, disproportionate dwarfism and impaired mental development (cretinism) are prominent signs of congenital and juvenile-onset hypothyroidism. With primary congenital hypothyroidism, enlargement of the thyroid gland (goiter) is also often observed. Puppies, kittens, and foals with this condition are behaviorally dull and less active, may have a shuffling gait, and have a poor appetite. On neurological examination, the animal is often weak, hyporeflexic, or hyperreflexic (if there is muscle tremor or spasticity), and it may lack conscious proprioception. Angular deformities have been observed in foals. Radiographic signs of underdeveloped

Table 28.4 Physiological effects of thyroid hormone. Source: Ferguson, 1989a. Reproduced with permission of Elsevier.

Site of action	Effect of hormone	Effects of deficit
Calorigenesis	Increase in BMR	Lethargy, weakness
Thermoregulation	O_2 consumption	Distal extremity hypothermia, heat-seeking
Growth and maturation	Normal CNS development	Mental retardation of cretin and dullness in adults; neuropathies
Carbohydrate metabolism	Increase in glycogenolysis and glycolysis, antiinsulin effects	Obesity despite normal or decreased appetite
Protein metabolism	Increased synthesis and degradation	Muscle weakness, poor hair coat and regrowth
Dermatological	Normal maintenance of anagen hairs, maintenance of fatty acid turnover in skin, normal keratin turnover rates	Bilateral symmetrical alopecia, hyperkeratosis, myxedema
Cardiovascular	Stimulation of myosin ATPase, stimulation of Na^+,K^+-ATPase, increased β-receptor numbers	Decreased heart rate, pulse, pressure, and cardiac output
Neuromuscular	Normal myelin production, maintenance of balance between slow-twitch and fast-twitch fibers	Polyneuropathy, muscle atrophy, weakness, stiffness, myotonia
Gastrointestinal	Maintenance of normal electrical activity of GI smooth muscle, normal segmentation	Diarrhea or constipation
Reproductive	Maintenance of normal protein synthetic rates	*Female:* anestrus, irregular cycles, galactorrhea, stillbirth *Male:* azospermia, lack of libido
Immunological	Stimulus of humoral and cell-mediated immunity	Recurrent infections (especially pyodermas)
Hematological	Bone marrow stimulation, factor VIII and VIIIAg production, normal platelet synthesis and function	Nonresponsive anemia, possible bleeding tendency
Endocrine	Normal secretion of growth hormone, gonadotropins, cortisol; inhibition of secretion of prolactin	Secondary growth hormone deficiency, galactorrhea

BMR, basal metabolic rate; CNS, central nervous system; GI, gastrointestinal.

epiphyses, shortened vertebral bodies, and delayed epiphyseal closure are common.

Effects on Lipid and Carbohydrate Metabolism

Thyroid hormone increases gluconeogenesis and glycogenolysis, contributing to its insulin antagonistic properties. Cholesterol synthesis and degradation are both increased by thyroid hormones and mediated by an increase in hepatic low density lipoprotein (LDL) receptors. Therefore, hypercholesterolemia is a common finding in hypothyroidism. Thyroid hormones stimulate lipolysis, releasing nonesterified fatty acids (NEFAs) and glycerol. Obesity may develop in some hypothyroid animals despite a normal appetite and caloric intake. In a study of lean and obese cats, T_3 administration increased thermogenesis and NEFA concentrations, as well as mRNA for adipose tissue peroxisome proliferator-activating receptor γ (PPARγ) and D1 in lean cats (Hoenig et al., 2008).

Dermatological Effects

Thyroid hormones in physiological quantities are necessary for normal hair and skin turnover. Thyroid insufficiency results in an increased percentage of telogen (inactive) hair follicles and an increase in keratin and sebum production. Dryness of the hair coat, excessive shedding, and retarded regrowth of hair are early signs of hypothyroidism in dogs. Alopecia, present in about two-thirds of affected dogs, is usually bilateral and symmetrical in distribution, and is most obvious over points of friction, such as the ventral trunk and neck, and axilla, and tail (“rattail” appearance) but also is common in the perineal area, and the dorsum of the tail and nose. The alopecia is classically nonpruritic unless secondary seborrhea or dermatitis has developed. Thickening of the skin and/or the development of myxedema (subcutaneous accumulation of glycosaminoglycans), develops in some cases. Myxedema is most prominent in the facial features, which may take on a puffy or “tragic” appearance. The type and distribution of dermal fatty acids can even be stimulated by replacement dosages of thyroid hormone in euthyroid animals. It is possible that this effect is truly a pharmacological effect of thyroid hormones and might explain the improvement in hair coat some dogs experience following thyroid hormone administration even when diagnostic tests fail to confirm hypothyroidism (Campbell and Davis, 1990).

Cardiovascular Effects

The major physiological effects of thyroid hormone on the myocardium are: (i) a direct positive inotropic effect, (ii) stimulation of myocardial hypertrophy, and (iii) increased responsiveness to adrenergic stimulation. Thyroid hormones increase the sarcolemmal Na^+,K^+-ATPase activity and favor the transcription of the α or "fast-twitch" form of the cardiac myosin ATPase improving cardiac contractility. In addition, myocardial contractility is improved by increasing the number of L-type calcium channels and enhancing sarcoplasmic reticulum calcium uptake and release. Thyroid hormones increased the number of β-adrenergic receptors in the heart, skeletal muscle, adipose tissue, and lymphocytes. In hyperthyroidism, tachycardia often results from this mechanism. Thyroid hormones also decrease α-adrenergic receptors in cardiac and vascular tissue. In hypothyroidism, the sensitivity to catecholamines in the peripheral vasculature is increased and may lead to peripheral hypothermia.

In dogs with hypothyroidism examined by an ECG, there is evidence of left ventricular dysfunction based upon cardiac echocardiography and electrocardiography. Following treatment with a replacement dosage of L-T_4 (0.5 mg/m^2 q 12 h) for 2 months, a significant decrease in shortening fraction and velocity of circumferential fiber shortening, as well as an increase in left ventricular end-systolic diameter, and prolongation of preejection period were noted when comparing measurements before and after levothyroxine supplementation. On electrocardiography, P and R wave amplitudes were significantly higher after treatment than before. Therefore, changes in cardiac function during hypothyroidism can be reversed (Panciera, 1994). Despite this, administration of the same dosage of L-T_4 to euthyroid dogs did not lead to any alteration of echocardiographic or electrocardiographic measurements (Panciera and Post, 1992). However, the majority of cats with spontaneous hyperthyroidism have echocardiographic evidence of cardiac hypertrophy and electrocardiographic abnormalities (Bond et al., 1988; Kienle et al., 1994; Fox et al., 1999).

Neuromuscular Effects

Thyroid hormones stimulate the synthesis of many proteins associated with normal nerve and muscle activity. For example, nerve Na^+,K^+-ATPase and fast forms of the myosin ATPase in muscle are stimulated by thyroid hormone. Myopathies have also been associated with hypothyroidism in domestic animals. Severe muscle weakness and delayed reflexes may be the clinical manifestation, or the signs may be vague such as stiffness, reluctance to move, and muscle wasting. Facial muscle and eyelid weakness (lip and lid droop) attributable to cranial nerve VII paralysis or paresis have been observed in dogs. Also, head tilt may be observed consistent with vestibular nerve disruption. These changes are likely due to the swelling of and around the dural sheath of the facial, vestibular, and cochlear nerves as they pass through bony foramina in the facial bones. Bilateral laryngeal paralysis has been associated with hypothyroidism in dogs as well. The pathophysiology of polyneuropathies associated with hypothyroidism is poorly understood but may be due to altered neuronal metabolism. Segmental demyelination and axonopathy has also been shown. Alternatively, compressive neurological abnormalities may be the result of tissue swelling (myxedema) surrounding the spinal cord or peripheral nerve. Clinically and electrodiagnostically, the polyneuropathy is indistinguishable from those caused by other diseases with hyporeflexia, slow nerve conduction velocities, fibrillation potentials, and positive sharp waves on electromyography. Although extremely rare, central nervous system (CNS) signs of seizures, disorientation, and circling also have been reported in hypothyroid dogs with cerebrovascular atherosclerosis caused by the hyperlipidemia associated hypothyroidism. Severe mental obtundation can be also observed in the syndromes of cretinism and myxedema coma.

Gastrointestinal Effects

Studies in hypothyroid dogs have demonstrated a decrease in the intestinal and gastric electrical and motor activity. Although hypothyroid dogs usually have normal bowel movements, constipation and diarrhea have been also observed.

Reproductive Effects

Normal thyroid hormone concentrations appear to be important for normal reproductive cycling of mammals. Hypothyroidism has been associated with a variety of reproductive disturbances in dogs and horses. In breeding bitches, persistent or sporadic anestrus, infertility, abortion, and high puppy mortality have been observed. Galactorrhea is a rare sign of hypothyroidism that develops in some intact female dogs whose mammae have been primed for lactation. Hyperprolactinemia, perhaps resulting from the excessive stimulation of prolactin-secreting pituitary cells by TRH, appears to be the cause of galactorrhea in susceptible bitches and may be at least partially responsible for the infertility associated with canine hypothyroidism. Lack of libido, testicular atrophy, hypospermia, or infertility have been suspected in the male with hypothyroidism, but studies of thyroidectomized dogs showed no changes in sperm count or motility. It is possible that there are components of autoimmune orchitis coexisting with autoimmune thyroiditis in the spontaneously developing form of hypothyroidism.

Immunological Effects

Any dog with a recurrent infection, particularly of the skin, should be evaluated for hypothyroidism. Pyoderma, which is unresponsive or only temporarily responsive to appropriate antibacterial agents, may be exacerbated by the reduced phagocytic function of white blood cells in hypothyroidism.

Hematological Effects

The increased cellular demand for oxygen stimulated by thyroid hormone leads to increased production of erythropoietin and increased red blood cell production by the bone marrow. Thyroid hormones also increase the 2,3-diphosphoglycerate content of erythrocytes, allowing increased oxygen dissociation from hemoglobin and increased availability to tissues.

A cause-and-effect relationship between canine hypothyroidism and the development of an acquired coagulation defect (von Willebrand's disease) has been postulated, but controlled studies in hypothyroid dogs have not confirmed a relationship. Similarly, in dogs with von Willebrand's disease treated with thyroid hormone, a rise in factor VIII antigen has been described even when little evidence of primary hypothyroidism exists. The mechanism of action of thyroxine in these circumstances is uncertain, but may reflect the nonspecific action of thyroid hormone on protein synthesis. Since the breed incidence of von Willebrand's disease and hypothyroidism overlap (e.g., Doberman Pinscher, Golden Retriever, Miniature Schnauzer), it is critical to rule out the coexistence of these conditions in individual dogs where hypothyroidism may unmask a subclinical bleeding tendency. Platelet number and function can be decreased in hypothyroidism.

Endocrine Effects

Thyroid hormones influence the normal secretion and metabolism of a variety of hormones and xenobiotics. Secretion of growth hormone, gonadotropins, and cortisol are stimulated by thyroid hormones and prolactin secretion is inhibited. Hypothyroidism may cause galactorrhea due to the subsequent increase in prolactin secretion in this condition (Braverman and Utiger, 1991; Burrow et al., 1989; Ferguson, 1989a, 1993; Peterson and Ferguson, 1990; Ferguson and Hoenig, 1991b; Greenspan, 1994; Panciera and Johnson, 1994, 1996; Johnson et al., 1999).

Thyroid Hormone Preparations

Thyroid hormone preparations can be classified into the following groups: (i) crude hormones prepared from animal thyroid gland; (ii) synthetic L-thyroxine (L-T_4); and (iii) synthetic L-triiodothyronine (L-T_3), and combinations of T_4 and T_3. The available products and dosage ranges are listed in Tables 28.5 and 28.6. The American Thyroid Association task force on thyroid hormone replacement has extensively reviewed the physiological principles and human clinical data regarding rationale and efficacy of thyroid hormone replacement products (Jonklaas et al., 2014). Many of these principles apply to veterinary applications as well and are reiterated here.

Crude Thyroid Products

Thyroid hormone products derived from thyroid tissue from hog, sheep, or cattle are available in the forms of desiccated thyroid (thyroglobulin). There are no good reasons to continue to use these products for replacement therapy in small animals; however, due to decreased cost, these products still have some utility in large animals. Problems with desiccated thyroid products include a highly variable content of T_4 and T_3, unphysiologically low ratios of T_4/T_3 (2 to 4), and short shelf life; these drawbacks outweigh the lower cost of these products. Standards set by the US Pharmacopoeia for control of hormone content may have improved the reproducibility of these products but are unlikely to eliminate the other disadvantages. However, a double-blind study in human patients compared desiccated thyroid to synthetic levothyroxine. Desiccated thyroid extract use led to greater weight loss, but there was no difference in psychometric testing or in any symptoms, and both products normalized hormonal concentrations. Both types of thyroid hormone were able to normalize the abnormal thyroid blood tests (Hoang et al., 2013).

Synthetic L-Thyroxine

Levothyroxine (L-T_4) is the thyroid hormone replacement compound of choice in all species. It is generally formulated and used as levothyroxine sodium for oral administration. Injectable forms are also available (for the rare indication of myxedema coma). Thyroxine is recommended for the following reasons:

1) L-T_4 is the main secretory product of the thyroid gland.
2) L-T_4 is the physiological "prohormone"; administration of L-T_4 does not bypass the cellular regulatory processes controlling the production of the more potent T_3 from T_4 (5′-deiodination).
3) In human patients with untreated hypothyroidism, serum TSH concentrations correlate inversely with serum free T_4 concentrations but to a lesser extent with serum T_3 concentrations. However, as mentioned previously, there is some evidence that there might be some clinical benefits associated with administration of a small amount of T_3 in humans,

Table 28.5 Levothyroxine products (all products are oral unless otherwise designated)

Product	Manufacturer	Starting dosage and frequency
Approved for Veterinary Use		
Thyro-Tabs Canine[a]	Lloyd	0.022 mg/kg q 24 h
Available but not FDA-approved for equine use[b]		
Thyro-L Equine Powder	Lloyd	0.01–0.1 mg/kg q 24 h or divided q 12 h; do not exceed 100 mg/horse/day
Thyroxine-L Powder	Allivet Pet Pharmaceuticals	
Thyrovet Equine Powder	Allivet Pet Pharmaceuticals	
Thyrozine Powder	Phoenix	
Approved for human use but available for veterinary use		
Levolet	Vintage (not marketed in the USA)	Note: for all products below and unapproved veterinary products on market: For dogs and cats, 0.022–0.044 mg/kg q 24 h or divided q 12 h may allow for variation in oral bioavailability; exceed only with caution 1 mg/day in dogs and 0.2 mg/day in cats
Levo-T	Alara Pharm	
Levothroid (oral and injectable)	Lloyd (distributed by Actavis)	
Levothyroxine Sodium	Genpharm	
Levothyroxine Sodium	Mylan	
Levoxyl	Jones Pharma/King	
Synthroid (oral and injectable)	AbbVie	
Tirosint	Institute Biochimique	
Unithroid	Jerome Stevens	

*[a] In January, 2016, the FDA issued letters to six manufacturers of the unapproved levothyroxine products stating “Unapproved animal drugs have not been reviewed by FDA and may not meet the agency’s strict standards for safety and effectiveness. They also may not be properly manufactured or properly labeled. FDA advises veterinarians and dog owners to avoid using unapproved levothyroxine products.” The warning letters targeted these unapproved levothyroxine products: Thyrosyn, Soloxine, Levocrine, Thyroxine L, Thyrovet, Thyromed, Thyroid Chewable Tablets, Thyrokare, Leventa. (https://www.fda.gov/animalveterinary/newsevents/cvmupdates/ucm482928.htm, January 29, 2016).
As of publication date, the only approved veterinary product for levothyroxine is Thyro-Tabs^{TM} (Lloyd, Inc.). Other products appear to have been removed temporarily or permanently from manufacture and additional distribution. Veterinarians should contact manufacturers directly for the most current status of these products. Some of these products may also still be distributed outside the USA (https://www.fda.gov/AnimalVeterinary/Products/ApprovedAnimalDrugProducts/UCM042860.pdf, accessed March, 2017).
†[b] There are no approved levothyroxine supplements for horses or cats.

Table 28.6 L-Triiodothyronine, combination levothyroxine/L-triiodothyronine and dessicated thyroid products approved by FDA for human use (oral unless otherwise designated)

Product	Manufacturer	Dosage and Frequency
L-Triiodothyronine		
Cytomel	Pfizer	Dogs: 0.004–0.006 mg/kg q 8–12 h Cats (T3 suppression test): 0.025 mg q 8 h × 7 doses
Liothyronine Sodium	Mylan	Dogs: 0.004–0.006 mg/kg q 8–12 h Cats (T3 suppression test): 0.025 mg q 8 h × 7 doses
Triostat (injectable)	JHP Pharmaceuticals, X-Gen Pharmaceuticals	Not available for dogs, cats, or horses
Levothyroxine/L-triiodothyronine		
Thyrolar (4 : 1 T4/T3 ratio)	Actavis	Based upon T4 dosage; see above
Dessicated thyroid		
Armour Thyroid	Allergan	Dosage not available for dogs and cats; 15 grains/day q 24 h for horse
Nature Thyroid	RLC Laboratories	Dosage not available for dogs and cats; 15 grains/day q 24 h for horse

and studies in rodents have long shown that normalization of TSH values with T_4 monotherapy often results in T_4 values, which are in the high normal or high range.

4) The therapeutic goal should be to normalize both tissue and serum T_4 and T_3 concentrations, and this is accomplished only in tissues with exogenous T_4 administration.
5) The central nervous system and pituitary derive a large proportion of intracellular T_3 from local 5′-deiodination of T_4 by D2. With administration of T_3, the serum T_3 concentrations must be higher than normal to normalize serum TSH (Figure 28.7).
6) In general, variability in the bioavailability of synthetic T_4 preparations is less than for the crude products.
7) L-T_4 is less expensive than other synthetic preparations.

Quality Control of Thyroid Hormone Products

In 1982, the US Pharmacopoeia adopted a new method for the assay of hormone content in thyroid hormone preparations. The old, less accurate determinations based upon iodine content were replaced by high pressure liquid chromatographic (HPLC) determination. Initially, studies of brand-name and generic L-thyroxine preparations showed that the hormonal content of some generic tablets may be as little as 30% of the amount stated on the label. The problems with variable hormone content have largely been addressed by the new standards. However, it is not safe to assume that the relative bioavailability of one thyroid hormone preparation is equivalent to another. Therefore, when starting an animal on a thyroid replacement product, it is recommended to start with a brand-name product (or proven generic product) with which broad experience has been obtained and use this product until a distinct clinical response has been seen. If no response is seen at a reasonable dose after a period of at least 4–6 weeks, and normal serum T_4 concentrations are achieved after administration, the diagnosis should be reevaluated. Except for financial reasons, the concern about mild overreplacement is minimal in most cases since the dog (as well as the cat) is very resistant to the development of thyrotoxic signs, requiring 10 to 20 times the replacement dose chronically in order to demonstrate signs. This is likely the result of the dog's and cat's capacity to efficiently clear thyroid hormone via biliary and fecal excretion (Ferguson, 1986; Kaptein et al., 1994).

Dosage Considerations for L-Thyroxine

Thyroid hormone replacement therapy is almost always indicated for the remainder of the dog's life. Therefore, careful initial diagnosis and tailoring of treatment is essential. A variety of dosage regimens for T_4 therapy have been recommended. This probably reflects the variation between animals in hormone absorption and metabolism, the variable degree of remaining endogenous hormone secretion by the failing thyroid, the possible effect of circulating anti-T_4 antibodies in a subgroup of animals, the resistance to the development of thyrotoxicosis with overdoses in the dog, and the vague and variable criteria by which clinical improvement is judged. With the advent of the canine TSH assay, objective analysis of the body's response (at least the pituitary's) to

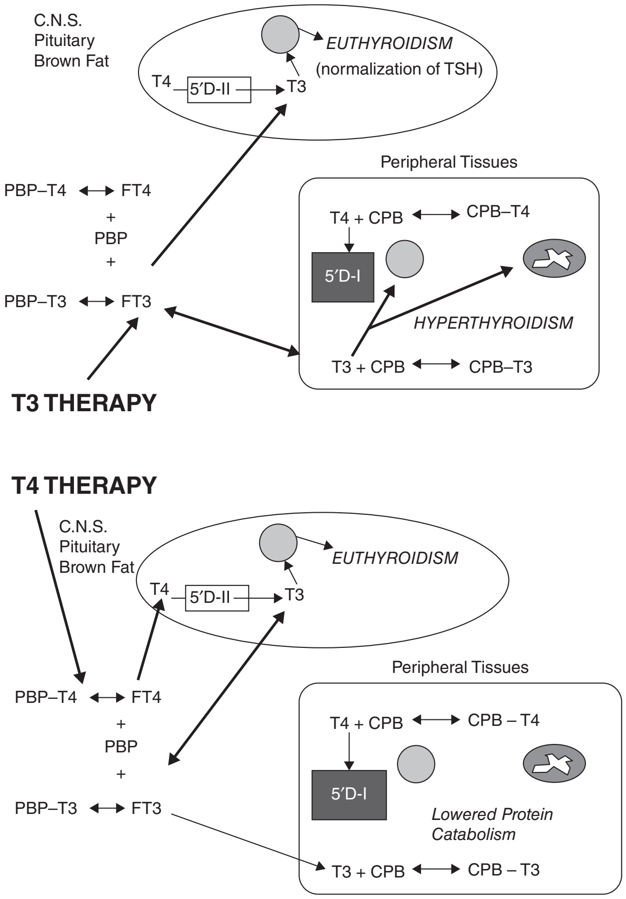


Figure 28.7 Thyroid replacement therapy of hypothyroidism with L-T_3 (*top*) or L-T_4 in supervening nonthyroidal illness. *Top*, L-T_3 therapy during nonthyroidal illness bypasses the individual tissue regulation of the 5′-deiodinase enzymes. Shown is the scenario when the amount of T_3 administered is sufficient to recreate euthyroidism in the pituitary, brown fat, or CNS. In the pituitary, TSH secretion would be reduced to normal. This amount of T_3 would be excessive for tissues such as the liver and kidney, which are trying to limit protein catabolism during illness. A state of tissue hyperthyroidism results. *Bottom,* In contrast to therapy with L-T_3, L-T_4 therapy allows individual tissues to regulate T_3 production. Therefore, the brain, pituitary, and brown fat continue to produce adequate amounts of T_3 derived from plasma T_4, but the liver, kidney, and other tissues reduce local T_3 production, allowing a lowering of protein catabolism in illness. PBP, plasma binding protein; CBP, cytosolic binding protein; FT_3, free triiodothyronine; FT_4, free thyroxine. Source: Peterson and Ferguson, 1990. Reproduced with permission of Elsevier.

exogenous hormone replacement is now possible and is routinely used in the monitoring of human patients with hypothyroidism. Using the FDA-unapproved veterinary L-thyroxine product Soloxine^{TM}, serum TSH concentration was suppressed to undetectable concentrations by 0.02 mg/kg or less of L-T_4 given only once daily (Ferguson, 2007).

In addition to the aforementioned effects of concomitant drug therapy on thyroid hormone metabolism, increased doses of T_4 appear to be necessary in hypothyroid humans during the colder months of winter. While similar studies of L-T_4 dosage have not been performed in animals, seasonal variations have been seen in dogs exposed to significant seasonal changes in light, as in racing dogs in Alaska. It is possible that an animal housed outdoors might require a higher dose of T_4 than an animal predominantly in the house, particularly during the colder months. In the dog, as in humans, basal serum concentrations of T_4 decrease with age. It has been observed in humans that older hypothyroid patients require lower doses of T_4 for adequate replacement, and are more apt to develop the adverse effects of slight thyroid hormone overdoses (Ferguson, 1986; Rosychuk, 1982).

Levothyroxine Dosages for the Dog

Based upon isotopic kinetic studies, L-thyroxine is produced and degraded at the rate of 7 μg/kg/day in dogs (Table 28.2) (Kaptein et al., 1993, 1994). In general, reported oral replacement doses for T_4 in the dog range from a total dose of 0.02 to 0.04 mg/kg daily. Based upon this indirect evidence and unpublished studies by the author of the oral bioavailability of common brands of levothyroxine, the fraction of oral absorption of L-T_4 products may range from 10 to 50%, averaging 35% (Ferguson and Hoenig, 1997), in part explaining the variation of necessary oral dosage to attain clinical euthyroidism. Therefore, if the daily production rate of T_4 is 7 μg/kg/day, and average bioavailability is 35%, a total daily dosage of 20 μg/kg or 0.02 mg/kg is predicted. In a study of a commercial liquid levothyroxine product, the dosage of 20 μg/kg once daily resolved clinical signs in 91% of dogs after 4 weeks of therapy. Following clinical signs, and total T_4 and TSH concentrations, once-daily maintenance dosages at steady-state were 20 μg L-T_4/kg BW for 79% of the dogs, 30 μg/kg for 15%, and 10-15 μg/kg in 6% (Le Traon, 2009). In addition, serum TSH concentrations were suppressed for at least 24 hours (Le Traon, 2009). A later study of chronic therapy demonstrated that only one out of 10 hypothyroid dogs required twice-daily oral replacement dosing to maintain clinical response (van Dijl et al., 2014).

Pharmacokinetic studies of a levothyroxine solution product in healthy dogs demonstrated 22% bioavailabiity with a maximum concentration at 3 hours postdosing, and half-life of 11.6 hours. Food intake concomitant with L-T_4 oral administration delayed L-T_4 absorption and decreased its rate and extent by about 45%. The relative bioavailability of L-T_4 following administration of a tablet formulation was about 50% of that of the L-T_4 solution. The pharmacokinetic properties of an oral L-T_4 solution support the use of a dose rate of 20 μg/kg once daily as a starting dose for replacement therapy in dogs with hypothyroidism (Le Traon et al., 2008). Although bioavailability of this same product was reduced 45% by concomitant feeding (Le Traon et al., 2008), the composition of the food (protein, fiber, etc.) does not seem to impact the resulting bioavailability (Iemura et al., 2013).

It has also been proposed that the dosage might be calculated according to body surface area (BSA) (0.5 mg/m^2) with the reasoning that it is proportional to the metabolic rate. When the L-T_4 dosage is determined on a body weight basis, large breed dogs have a greater tendency to develop thyrotoxicosis, or at least elevated serum T_4 concentrations. For example, according to body surface area, a 5-kg (0.29 m^2 BSA) dog would start on a dose of 0.15 mg L-T_4 daily, or 0.03 mg/kg. A 50-kg dog (1.36 m^2 BSA) would start on 0.70 mg/day, or less than half the dose according to body weight, 0.014 mg/kg. There are no published experimental studies, however, to confirm the validity of this dosing method. It is rarely necessary to exceed 1 mg of levothyroxine per day in dogs, but some experts place the upper dosage at 1.6 mg per day (Ferguson, 1986; Ferguson and Hoenig, 1991b; Ferguson et al., 1992; Rosychuk, 1982).

As thyroid replacement therapy is initiated, a common practice is to divide the total daily dosage into two separate doses given at 12-hour intervals. Because of the significant intracellular capacity for storage of T_4, particularly in rapidly exchanging pools like liver and kidney, the initial oral doses of thyroid hormone may be substantially distributed into tissue stores, the hormone stores of the liver and kidney are repleted to euthyroid levels, and then can serve to "buffer" serum concentrations when the circulating hormone "store" bound to binding proteins are depleted. As previously outlined, hypothyroidism reduces the deiodinative and conjugative rate of thyroid hormone metabolism. Division of the daily dose reduces the metabolic effect of a bolus of thyroid hormone on hypothyroid tissues and decreases the "one-pass" effect (i.e., hepatic metabolism and excretion of a portion of a bolus dose of hormone before ever reaching the systemic circulation). In the rat, the largest compartment for thyroid hormone has been shown to be the gastrointestinal tract. The clinical result is that many hypothyroid animals can be maintained on once-daily T_4 therapy despite the fact that the serum half-life is much shorter. In humans, it seems that clinical improvement and suppression of serum TSH can be maintained by any replacement regimen that, over the course of a day, leads to a normal average serum concentration without leading to the acute toxic effects of thyroid hormone. Although the serum T_4 concentration might be high at one time of the day and low at another, the tissue response "integrates" the serum concentration throughout the day, thereby reflecting the average concentration. Once-daily administration also leads to greater compliance by owners. In an animal that has responded to twice-daily therapy, the reappearance of clinical signs of hypothyroidism on a once-daily regimen should be a signal to return to the successful twice-daily regimen. Because the metabolism of thyroid hormone changes with correction of hypothyroidism, dosage regimes should be reassessed by clinical and laboratory criteria after at least 4 weeks of initial therapy.

Levothyroxine Dosages for the Cat

As in the dog, the recommended treatment for feline hypothyroidism is daily administration of L-thyroxine, using an initial dose of 0.05–0.1 mg twice daily (Peterson, 2013). This dosage should subsequently be adjusted on the basis of the cat's clinical response and post-pill serum T_4 evaluation (as described in Section Monitoring Therapy). Complete resolution of clinical signs can usually be expected in cats with adult-onset iatrogenic hypothyroidism. However, the mental dullness and dwarfism that develops in kittens with hypothyroidism usually persist because of delayed period of time from onset to diagnosis in these cats (Peterson and Ferguson, 1990; Rosychuk, 1982). The use of levothyroxine therapy following surgery or radioiodide therapy to treat resulting hypothyroidism has been shown to reduce the severity of post-treatment azotemia (Williams et al., 2014).

Levothyroxine Dosages for the Horse

There is little published information establishing therapeutic criteria for levothyroxine dosing in the horse. The oral L-T_4 dosage required clinically appears to depend largely on the form of hormone (crude versus synthetic) used for replacement therapy. The recommended starting dosage of levothyroxine has been 20 μg/kg/day orally (Frank et al., 2002). Higher dosages have been tolerated for short periods, but likely because it was given with grain, as fiber or bran may decrease oral absorption in humans (Breuhaus, 2002). Iodinated casein contains approximately 1% T_4 and is given at 5 to 15 g/horse/day orally (Lowe et al., 1974).

Synthetic L-Triiodothyronine

Although T_3 is the active intracellular hormone, there are few valid reasons to use this product for replacement therapy and some good reasons not to use it. Triiodothyronine therapy is not physiological as it bypasses the final cellular regulatory step of 5′-deiodination of T_4 (Figure 28.7). Thyroxine does have intrinsic thyromimetic activity. Its role is particularly important in the central nervous system and pituitary, tissues in which normalization of the intracellular T_3 concentration depends upon the normalization of both serum T_4 and T_3. Treatment with T_3 alone may provide amounts sufficient for organs like the liver, kidney, and heart, which derive a high proportion of T_3 from plasma. However, the brain and pituitary, which derive a majority of their T_3 from T_4 intracellularly, may then be deficient in thyroid hormone. Conversely, T_3 therapy adequate for the brain and pituitary may be excessive for the liver, kidney, and heart (Figure 28.7, top).

At present, it cannot be recommended that T_3 therapy be instituted in the "low T_3 syndrome" associated with nonthyroidal illness. Because of its higher oral bioavailability, it may be used to improve the clinical response in a dog with demonstrated or suspected poor T_4 absorption in which posttherapy serum T_4 and T_3 concentrations remain low despite increases in the oral daily T_4 dose. Triiodothyronine therapy may be indicated when thyroid replacement is necessary with the simultaneous administration of drugs, such as glucocorticoids, which inhibit the conversion of T_4 to T_3.

Anecdotal reports suggest that a small fraction of hypothyroid dogs convert T_4 to T_3 poorly in the absence of obvious nonthyroidal illness, and therefore do not respond to L-T_4 therapy. Triiodothyronine therapy has been recommended in these cases as an adjunct to T_4 or as sole therapy. The most likely cause of apparently low serum T_3 concentrations and normal or high T_4 concentrations following T_4 therapy is the presence of anti-T_3 antibodies that, in certain T_3 radioimmunoassays, will result an extremely low reading for the T_3 concentration. This observation is an in vitro artifact that has no relevance to the choice of replacement therapy products. Because the binding capacity of antithyroid hormone antibodies is easily overcome in most cases, it is recommended that usual dosages of L-T_4 be administered and the dose increased (if needed) until a clinical response is seen. The posttreatment serum T_3 concentration should be ignored in these dogs with T_3 autoantibodies (Ferguson, 1986; Rosychuk, 1982).

Combination T_4/T_3 Therapy

A study in humans compared the administration of solely T_4, with replacing 50 μg of the normal T_4 dose with 12.5 μg of T_3. Using neuropsychological methods for evaluation, the T_3-treated individuals showed improved cognitive performance (Bunevicius et al., 1999). This approach is controversial because other studies have not confirmed the finding. Similar studies have not been conducted in domestic animals.

Triiodothyronine Suppression Test in the Cat

As part of a workup for hyperthyroidism in cats, the administration of L-T_3 is utilized to evaluate the autonomy of thyroid secretion from the influence of pituitary TSH. L-triiodothyronine is administered at a dose of 25 µg every 8 hours for 2 days, giving a seventh dose on the morning of the third day. A blood sample for serum T_4 measurement is taken before T_3 administration and again at 4 hours after the last dose. Presumably mediated via a fall in pituitary TSH, serum T_4 is depressed by at least 50% in normal cats, whereas little suppression is seen in cats with hyperthyroidism because TSH is already depressed. The advantages of this test are that the doses of T_3 can be given at home on an outpatient basis with an office visit 4 hours after the last dose (Graves and Peterson, 1994; Peterson and Ferguson, 1990). The disadvantage is that there may be less than full compliance with successful administration of the seven doses of L-T_3 by the owner.

Effects of Thyroid Hormone Overdose (Iatrogenic Thyrotoxicosis)

Except for financial reasons, the concern about mild overreplacement is minimal in most cases as the dog is very resistant to the development of thyrotoxic signs. This resistance to iatrogenic thyrotoxicosis is the result of the dog's capacity to efficiently clear thyroid hormone via biliary and fecal excretion. Animals on replacement therapy, particularly with a T_3-containing product, can develop signs of thyrotoxicosis, particularly in large breed dogs dosed on a body weight basis; however, the incidence at recommended doses is rare. Animals should be monitored for signs suggesting an overdose including polyuria, polydipsia, nervousness, weight loss, increase in appetite, panting, and fever. Diagnosis is confirmed by elevated serum T_4 and/or T_3 concentrations and the amelioration of signs by temporary discontinuation of therapy. Following accidental ingestion of massive amounts of levothyroxine, dogs should be treated with activated charcoal within several hours of ingestion, possibly managed with β-adrenergic blockers if tachycardic, and heart rate and body temperature should be carefully monitored. Clinical experience has generally shown that dogs survive intoxication with few side effects despite considerable elevations in serum T_4 and T_3 concentrations.

Therapeutic Trial for Diagnosis of Hypothyroidism

Thyroid replacement therapy, without confirmatory laboratory evidence of hypothyroidism, has been suggested as a valid diagnostic step in a dog suspected to be hypothyroid. Although the major factor cited in defense of this practice is the cost of the diagnostic testing for the owner, it should be emphasized to an owner that replacement therapy is generally necessary for the remainder of the animal's life. Therefore, an incorrect diagnosis (and unnecessary long-term thyroid hormone treatment) can also be quite expensive. In one study of normal dogs given L-T_4 at the dosage of 0.5 mg/m^2 twice daily, the mean serum T_4 response to exogenous TSH had suppressed to 56% and 46% of the pretreatment value when retested at 4 and 8 weeks on treatment, respectively. Four weeks after cessation of L-T_4 therapy, the serum T_4 response to TSH was still slightly suppressed, indicative of residual thyroid atrophy (Panciera et al., 1990). Therefore, if TSH stimulation testing is used to confirm the diagnosis of hypothyroidism in a dog that has recently been receiving thyroid hormone, TSH testing should not be performed for at least 4 weeks following discontinuation of thyroid hormone replacement therapy.

Monitoring Therapy

The most important indicator of the success of thyroid replacement therapy is the progress made toward ameliorating clinical signs. Before therapy is begun, the clinician and owner should have a clear idea of the goals of therapy and the time frame in which these goals can reasonably be achieved. The reversal of changes in haircoat and body weight should be assessed no sooner than after 2 months of therapy. In cases in which clinical improvement is marginal or signs of thyrotoxicosis are seen, the clinical observations can be supported by therapeutic monitoring of serum thyroid hormone concentrations (post-pill testing). Clearly, the documentation of distinctly elevated serum T_4 concentrations following T_4 administration and elevated serum T_3 concentrations following T_3 administration, concomitant with signs of thyrotoxicosis, confirm an overdose. The interpretation of post-pill serum thyroid hormone concentrations in cases of suspected underdosing can be more complicated because the timing of sampling may be critical to the proper interpretation (Nachreiner et al., 1993). Ideally, therapeutic monitoring should not be attempted until steady-state conditions are reached, minimally 1 week after the initiation of therapy from a pharmacokinetic standpoint, but probably 1 month after initiation of therapy from a pharmacodynamic and clinical standpoint. With once-daily T_4 administration, the peak serum concentrations of T_4 generally should be in the high-normal to slightly high range 4–8 hours after dosing and should be low-normal to normal 24 hours after dosing. Given the dog's resistance to signs of thyrotoxicosis, it may be reasonable and adequate to check the serum T_4 concentration 24 hours after the previous day's dose. This is the method of choice of the author. In this situation, serum T_4 concentrations should still be in the normal range. Some endocrinologists prefer to measure "peak" T_4

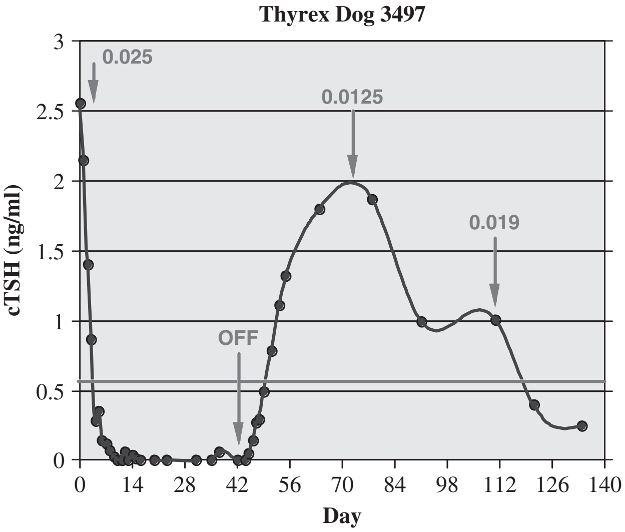


Figure 28.8 Effect of L-T_4 administration to a thyroidectomized dog on serum TSH concentrations. The numbers next to the arrows represent the dosage of L-T_4 administered once daily in mg/kg. Serum samples were taken 24 hours after the last dosing. On day 42, L-T_4 administration was terminated until day 70. The horizontal line represents the upper limit of the normal range for serum TSH concentration. Note the manner in which the varied L-T_4 dosage titrates the serum TSH concentration demonstrating the negative feedback system on the hypothalamus and pituitary.

concentrations at 4 or 6 hours after once-daily dosing, and some measure at "peak" and "trough" times. Animals on twice-daily administration probably can be checked at any time, but peak concentrations can be expected at the middle of the dosing interval (4–8 hours) and the nadir just prior to the next dose. Once the dog's dose is stabilized, once- or twice-yearly checks of serum T_4 (with or without T_3) concentrations are recommended.

Monitoring L-T_4 therapy may also be accomplished by documenting suppression of serum TSH. With the current generation of TSH immunoassays, concentrations are elevated only in about 75% of cases. Nonetheless, detectable TSH concentrations within the normal range still appear sensitive to titration of T_4 dosage (Figure 28.8). Another study demonstrated that serum TSH concentrations are suppressed for at least 24 hours following a single dose (Le Traon et al., 2009). Ninety percent of dogs evaluated chronically were managed successfully with once-daily dosing (van Dijl et al., 2014).

In a study of treatment of thyroidectomized dogs, it was shown that dosages as little as 0.02 mg/kg once a day will almost always suppress endogenous TSH concentrations into the normal or undetectable range. This observation establishes that the biological half-life of thyroid hormone exceeds by far the serum half-life (Ferguson and Hoenig, 1997). However, although titration of L-T_4 dosage by monitoring endogenous TSH concentrations is standard practice in hypothyroid human patients, no study has yet evaluated whether TSH suppression correlates consistently with a satisfactory clinical response in dogs. Therefore, it appears clear that dosing protocols recommending the dosage of 0.02 mg/kg twice daily probably reflect the dosage necessary for dogs with the lowest T_4 bioavailability. Unfortunately, the assay sensitivity does not allow the distinction of normal values from low values, so establishment of overtreatment and hyperthyroidism is not yet possible.

Although not recommended routinely, serum T_3 measurements following L-thyroxine administration should be interpreted together with the serum T_4 results and, most importantly, the clinical response. Low serum T_3 and T_4 concentrations, together with a poor clinical response, suggest an underdose or inadequate bioavailability (absorption). With T_3 administration, serum concentrations are reported to peak 2–3 hours after administration. Serum T_4 concentrations are routinely low or undetectable in dogs receiving T_3 therapy. Any remaining endogenous thyroidal T_4 secretion will be inhibited because of the suppression of pituitary TSH secretion by T_3 (Ferguson, 1986; Peterson and Ferguson, 1990; Rosychuk, 1982).

Therapeutic Failure

If clinical signs of hypothyroidism remain despite the use of reasonable doses of thyroid hormone, the following possibilities must be considered: (i) the dose or frequency of administration is improper; (ii) the owner is not complying with instructions or is not successfully administrating the product; (iii) the animal may not be absorbing the product well, or is metabolizing and/or excreting it too rapidly; (iv) the product is outdated; or (v) the diagnosis is incorrect. A syndrome of tissue resistance to thyroid hormone, while described in humans, has not yet been documented in the dog or cat.

Treatment of Myxedema Coma

Myxedema coma is a rare condition in domestic animals, only described in dogs, and is caused by severe untreated hypothyroidism. Often induced by an anesthetic episode or concurrent infectious disease, it results in severe mental obtundation or coma, and hypothermia. Common physical examination abnormalities include obesity and nonpitting edema (myxedema), and anemia and hypercholesterolemia are clinicopathological findings. Because of the extremely high mortality associated with this condition, it is essential that treatment be instituted promptly and vigorously as soon as the diagnosis is made. Treatment should include an intravenous dose of L-T_4 prepared for injection (100–200 µg total dose or 4–5 µg/kg), passive rewarming (wrapping in blankets, etc.), and mechanical respiratory support as needed.

Therapy for shock must include glucocorticoids and fluid and electrolyte replacement. Oral thyroxine therapy can be instituted when the animal stabilizes. A study of seven severely hypothyroid dogs treated with 4–5 μg/kg of L-T_4 IV observed improvement in mentation or ambulation and systolic hypotension within 30 hours of IV levothyroxine administration. Prognosis is good in most treated dogs (Pullen and Hess, 2006).

Antithyroid Drugs

Goitrogens

With the isolation and purification of thyroid peroxidase (TPO) enzyme of the thyroid gland, it became apparent that most compounds with antithyroid (goitrogenic) activity are inhibitors of TPO-catalyzed iodination. Plants of the genus *Brassica,* such as rutabaga, cabbage, and turnip, contain a compound called *goitrin* (see structure in Figure 28.9), which has antithyroid activity. In addition, plants such as broccoli and rapeseed include glucosinolates, which are metabolized to thiocyanate, an inhibitor of thyroid iodide uptake and organification. The so-called "cyanogenic glucosides," found in foods such as cassava, lima beans, and sweet potatoes, can be a source of cyanide, which then is detoxified to thiocyanate. Substituted phenols, such as resorcinol, phloroglucinol, and 2,4,dihydroxybenzoic acid, which may be found as contaminants in the water supply near coal conversion plants and may arise from degradation of flavonoids in plant material, also have goitrogenic activity (Taurog, 1991; Brucker-Davis, 1998; Cerundolo et al., 2009).

Studies have raised questions about the goitrogenic potential of environmental polyhalogenated aryl hydrocarbons (PHAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), plasticizer bisphenol A (BPA), and perchlorate on thyroid function. PHAHs have the particular potential to be retained by cats who have low glucuronyltransferase activity, and very high serum concentrations of PBDEs; dietary concentrations of BPA in cat food have been linked epidemiologically if not mechanistically to the development of feline hyperthyroidism. Confirmation of the link and the possible mechanisms still awaits further research (Edinboro et al., 2004; Dye et al., 2007; Mensching et al., 2007, 2012; Guo et al., 2012; Norrgran et al., 2015; Chow et al., 2015).

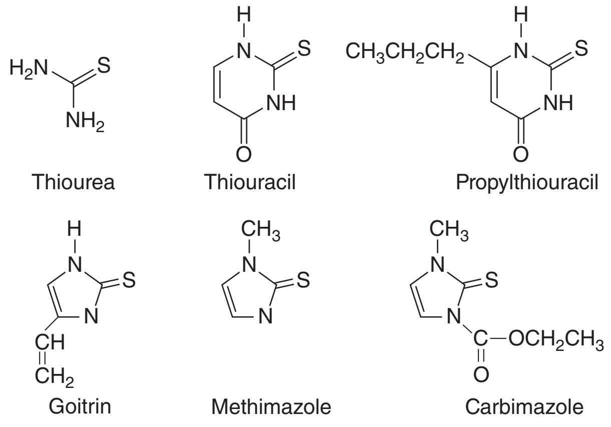


Figure 28.9 Thioureylene antithyroid drugs.

Thioureylenes or Thionamides

Antithyroid thioureylene or thionamide drugs are direct inhibitors of TPO, leading to a reduction in the organification and coupling steps of thyroid hormone synthesis. Figure 28.9 shows the structure of thiourea, goitrin, and the antithyroid drugs thiouracil, propylthiouracil, methimazole, and carbimazole. After administration, these drugs are actively concentrated by the thyroid gland, where they act to inhibit the synthesis of thyroid hormones through the following mechanisms: (i) blocking the incorporation of iodine into the tyrosyl groups in thyroglobulin; (ii) preventing the coupling of iodotyrosyl groups (mono- and diiodotyrosines) to form the ether linkage of T_4 and T_3; and (iii) direct interactions with the thyroglobulin molecule (Figure 28.1). Processes i and ii are mediated via inhibition of the enzyme TPO. Thionamide antithyroid drugs do not interfere with the thyroid gland's ability to concentrate, or "trap," inorganic iodine, do not block the release of stored thyroid hormone into the circulation, and do not damage the thyroid glandular tissue.

The thionamide drugs methimazole (MMI) and its prodrug carbimazole are currently the most commonly used antithyroid drugs in veterinary clinical practice (see Table 28.7 for products and dosages). Following initiation of treatment with MMI there is usually a slight delay in the fall of serum thyroid hormone concentrations as glandular hormone stores are becoming depleted. Another drug in this class, propylthiouracil (PTU), which has fallen out of favor due to autoimmune side effects in cats, has the additional mechanistic effect of blocking the conversion of T_4 to the more active T_3 in peripheral tissues like the liver and kidney. Therefore, serum T_4 may fall following both PTU and MMI therapy, but with MMI, due to autoregulatory mechanisms in the peripheral tissues (most likely up-regulation of D2 activity), serum T_3 is usually maintained within the normal range even when T_4 is quite low. As a result of this mechanism, it is rare to see a cat on MMI develop clinical signs of hypothyroidism. In some elderly hyperthyroid cats, elevations in serum creatinine with occasional overt renal failure develops. In cases where pretreatment renal function is in question, the use of MMI has gained favor because it provides a reversible and more gradual mode of therapy

Table 28.7 Antithyroid drugs

Drug	Product name	Dosage range and route (cats only)
Methimazole	Felimazole$^{\text{TM}}$; Tapazole$^{\text{TM}}$ (Jones Pharma), generic	Initial: 2.5–5 mg q 8–12 h PO Maintenance: 2.5–20 mg q 24 h or divided q 12 h
Carbimazole	Neomercazole$^{\text{TM}}$, Vidalta$^{\text{TM}}$, Neo-Carbimazole® zole®, Carbazole®, Neo Mercazole®, etc. (all only available in Europe and Canada)	Same as methimazole
Propylthiouracil	Propylthiouracil USP	50 mg q 8–12 h PO (generic)
Iopanoic acid	Telepaque$^{\text{TM}}$ (Amersham Health)	50 mg q 12 h PO (Note: dosage lowers serum T_3 only)
Potassium iodine (Lugol’s)		50 mg q 12–24 h PO

USP, US Pharmacopoeia.

for returning the animal to euthyroidism. It is now clear that the exacerbation of following therapy of hyperthyroidism is associated with posttreatment hypothyroidism identifiable by TSH concentrations above the normal range, observed in about one-third of cases in one study. Iatrogenic hypothyroidism was confirmed (low total T_4 and high TSH) in about 20% of cats. The incidence of increased creatinine concentrations was twofold higher (about 40%) in cats with elevated TSH compared to those with TSH within the reference range. The measurement of free T_4 by dialysis provided no additional information compared to measurement of total T_4 concentration (Aldridge et al., 2015). L-thyroxine supplementation helps to reduce exacerbation of azotemia in cats with hyperthyroidism and borderline renal disease (Broome et al., 2013).

Propylthiouracil

Propylthiouracil (PTU) is the prototype D1 inhibitor as well as being a TPO inhibitor. Despite its apparent additional therapeutic effects, the use of PTU has fallen out of favor because of its potential for serious side effects (Aucoin et al., 1985; Shenton et al., 2004). Like MMI, it can cause anorexia, vomiting, lethargy, and the development of positive antinuclear antibody titers, but has been associated with the development of autoimmune hemolytic anemia and immune-mediated thrombocytopenia. Because of the latter two complications, which are a particular problem in the animal being prepared for surgery, PTU can no longer be recommended for routine use in the hyperthyroid cat (Ferguson and Hoenig, 1991a; Ferguson et al., 1992; Kintzer, 1994; Peterson and Ferguson, 1990).

Methimazole

Methimazole is now the antithyroid medication of choice in the cat. The use of this drug in the cat has been well documented. In this study, doses as high as 5 mg q 8 h were applied, which reduced the serum T_4 into the normal range by 2–3 weeks. Since that time, most cats are diagnosed at an earlier phase of disease and MMI doses most commonly begin at 2.5 mg once or twice daily and are titrated upward in 2.5 mg (half tablet) intervals until the serum T_4 concentration falls within the lower half of the reference range. As mentioned, even cats with low serum T_4 may not become hypothyroid because the serum T_3 concentration generally remains normal. Once a satisfactory therapeutic effect is seen, many cats can be maintained on once-daily therapy, a major advantage for owner compliance. However, if a daily dose is missed, the serum T_4 concentration may rise rapidly. Cats on chronic MMI therapy should be checked at 1, 3 and 6 months to draw blood for serum T_4 measurement and to monitor for signs of drug toxicity (see below) (Peterson et al., 1988; Trepanier, 2007, 2015; Daminet et al., 2014).

Pharmacokinetics and pharmacodynamics of methimazole:
In euthyroid cats, the serum half-life has ranged from 4 to 6 hours following an IV dose. Following 2 weeks of oral methimazole, an average half-life of 3.4 hours was measured. In hyperthyroid cats, while the half-life was no different from euthyroid cats (2.3 hours), the mean residence time was shorter. Several studies in euthyroid and normal cats show bioavailability as averaging about 80%, with significant variability in bioavailability between animals. Studies in human hyperthyroid patients have shown that the drug has an intrathyroidal residence time of approximately 20 hours, which is fourfold longer than the serum half-life. Since antithyroid drugs act to inhibit thyroid hormone synthesis only after they are concentrated within the thyroid gland, serum half-life of these drugs may be of lesser importance than the intrathyroidal drug concentration for adequate control of the hyperthyroid state. There is usually a 1–3 week lag time between starting the drug and significant reductions in serum T_4 (Trepanier et al., 1991a, 1991b).

Adverse effects of methimazole:
Methimazole has been associated with the following adverse effects: anorexia (11.1%), vomiting (10.7%),

lethargy (8.8%), excoriations (2.3%), bleeding (2.3%), hepatopathy (1.5%), thrombocytopenia (2.7%), agranulocytosis (1.5%), leukopenia (4.7%), eosinophilia (11.3%), lymphocytosis (7.2%), positive antinuclear antibodies (ANA) (21.8%), and positive direct antiglobulin test (1.9%). The gastrointestinal adverse effects generally developed within the first month of treatment and usually resolved even with continued therapy.

Mild clinical side effects associated with methimazole treatment are relatively common (approximately 15% of cats) and include anorexia, vomiting, and lethargy. In most cats, these adverse signs are transient and resolve despite continued administration of the drug. Severe gastrointestinal signs persist in some cats, however, necessitating discontinuation of the drug. Self-induced excoriations of the face and neck also may develop in a few cats within the first 6 weeks of therapy. Although these cutaneous lesions tend to be partially responsive to treatment with systemic glucocorticoids, cessation of methimazole administration is usually required for complete resolution of these excoriations. Finally, hepatic toxicity is an uncommon but serious reaction that can develop during drug treatment. Methimazole-induced hepatopathy is characterized by the development of marked increases in serum concentrations of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (SAP), and total bilirubin. Clinical improvement, with resolution of anorexia, vomiting, and lethargy, usually occurs within a few days after cessation of methimazole, but jaundice and abnormal serum biochemical tests indicative of liver disease may not resolve for several weeks. Rechallenge with the drug will again induce clinical signs and serum biochemical abnormalities indicative of hepatic disease within a few days. A variety of hematological abnormalities may develop in cats during treatment with methimazole. Those abnormalities that do not appear to be associated with any adverse effects include eosinophilia, lymphocytosis, and transient leukopenia with a normal differential count. As with PTU treatment, more serious hematological reactions that develop in a few cats treated with methimazole include severe thrombocytopenia (platelet count <75,000 cells/mm^3) and agranulocytosis (severe leukopenia with a total granulocyte count <250 cells/mm^3). Most cats that develop severe thrombocytopenia also show concomitant overt bleeding (i.e., epistaxis, oral hemorrhage). Development of agranulocytosis during methimazole treatment predisposes to severe bacterial infections, systemic toxicity, and fever. If serious hematological reactions develop during methimazole therapy, the drug should be stopped and supportive care given; these adverse reactions should resolve within 5 days after the methimazole is withdrawn. Since most life-threatening side effects (e.g., hepatopathy, thrombocytopenia, agranulocytosis) caused by methimazole treatment usually again develop quickly after rechallenge with the drug, alternative therapy with either surgery or radioiodine should be considered in these cases.

During methimazole therapy, serum ANA develop in a high percentage of cats after treatment with methimazole. The risk of developing ANA appears to increase with the duration of methimazole treatment, with ANA developing in approximately half of cats treated for greater than 6 months. The risk of developing serum ANA also appears to be greater for cats treated with higher daily methimazole doses, since most cats that develop ANA are receiving doses ≥15 mg/day and ANA will disappear in most cats after the dosage is decreased. Despite the high prevalence of ANA development during long-term treatment with methimazole, clinical signs associated with a lupus-like syndrome (i.e., dermatitis, polyarthritis, glomerulonephritis, hemolytic anemia, or fever) have not been observed in any of these cats. The daily drug dosage should therefore be decreased to as low as possible (while still maintaining serum T_4 values within the low normal range), since ANA tests will become negative in many cats when the methimazole dosage is decreased (Peterson et al., 1988; Daminet et al., 2014; Trepanier, 2015).

Transdermal Methimazole

Methimazole compounded in a pluronic lecithin organogel (PLO) dosage form has begun to be offered by compounding pharmacists. Transdermal pluronic gels have been shown to modulate permeation of a variety of drugs from small organics to peptides. Lecithin is an emulsifying agent that increases fluidity of stratum corneum, and can result in exfoliation and low-grade inflammation, resulting in greater absorption when given chronically. Transdermal administration may be considered when oral therapy of MMI is impractical in a cat. Although a pharmacokinetic study initially showed poor to no bioavailability for methimazole formulated in PLO and administered as a single dose inside the pinna of healthy cats, studies of daily administration to hyperthyroid cats for 4 weeks have reported clinical response and reduction of serum T_4 concentrations. The time to optimal reduction of serum T_4 response to treatment tends to be slower than with oral therapy. Furthermore, the response is more variable, possibly because of greater variability in factors determining transdermal bioavailability. Despite questionable absorption in euthyroid cats following a single dose, a study by Sartor et al. (2004) evaluated the efficacy and safety of transdermal MMI in 47 hyperthyroid cats. A randomized clinical trial compared transdermal with oral MMI at 2.5 mg q 12 h for both routes. While 88% of cats treated by oral route had serum T_4 concentrations within the reference range after 2 weeks, 56% of transdermally treated cats reached this goal. This efficacy gap reduced to 80% with oral administration and 67% by transdermal administration (not significant). Oral MMI cats had a higher incidence of gastrointestinal (GI) adverse effects (24%) compared to the cats treated with transdermal methimazole (4%), but no differences were found between groups in the incidence of other side effects. In this study, the absolute bioavailability (compared to IV route) was 40% for the oral and transdermal routes. Another study of 13 hyperthyroid cats given 5 mg/0.1 ml of MMI q 12 h showed that total T_4 concentrations fell to 28% of baseline at 14 days and 15% of baseline (low normal range) at 28 days. The gastrointestinal side effects also appeared reduced with the transdermal route (Lecuyer et al., 2006). Another study of a new lipophilic formulation had about half the relative bioavailablity compared to a similar dose of carbimazole (see Section Carbimazole) (Hill et al., 2014).

More recent studies utilized a starting dose of 2.5 mg per cat q 12 h and 5 mg per cat q 24 h). Significantly decreased T_4 concentrations were observed in weeks 1 and 3. All cats showed sustained suppression of T_4 concentration during the 10-hour period in both daily and twice daily dosing regimens. As a result, sampling time for T_4 measurement does not seem to be critical. However, it seems difficult to keep the T_4 concentrations constantly within the reference interval. Median follow-up time was almost 2 years with daily doses ranged from 1 to 15 mg. Higher doses can be expected after prolonged treatment and, despite greater convenience for the owner, compliance should be regularly evaluated (Boretti et al., 2013, 2014).

Methimazole, Thyroid Scintigraphy, and Subsequent Ablative Radioiodide Therapy

Studies are conflicting about whether concurrent methimazole affects results of diagnostic scintigraphy. One study demonstrated that ^{123}I uptake is not inhibited by MMI treatment in normal cats, but is significantly increased after withdrawal with peak uptake between 4 and 9 days after discontinuation of MMI. Increased uptake of [$^{99m}TcO_4$] may also affect the interpretation of [$^{99m}TcO_4$] thyroid scintigraphy for 2–3 weeks (Nieckarz and Daniel, 2001). These scintigraphic findings are likely an artifact of seeing intense pertechnetate uptake in large hyperfunctional thyroid nodules, and then, upon reduction of T_4, the increase of TSH would stimulate the normal tissue or less active tissue to take up [$^{99m}TcO_4$.] Another study saw no effect, but also did not see TSH rise in most cats (Fischetti et al., 2005). Some hyperthyroid cats can be diagnosed by thyroid scan as having unilateral disease before MMI treatment, and then, once euthyroid on therapy, can appear on thyroid scan to have bilateral disease (Peterson and Broome, 2015).

Monitoring Patients on Methimazole

In addition to serum total T_4 or possibly even TSH concentrations, the following liver function tests are recommended: ALT, SAP, and/or AST, and total bilirubin. Alkaline phosphatase is likely high in hyperthyroidism because of an elevation of the bone isoenzyme associated with increased calcium turnover. Renal function tests including blood urea nitrogen (BUN) and creatinine concentrations are important in the first month of treatment to monitor the effect of correction of hyperthyroidism on reduction of glomerular filtration rate (GFR). Complete blood count (CBC) and platelet count should be periodically monitored as peak chance of autoimmune phenomena is at about 3 months of therapy (Peterson et al., 1988; Trepanier, 2007; Rutland et al., 2009; Daminet et al., 2014).

Carbimazole

The antithyroid drug carbimazole is a carbethoxy derivative of methimazole that is rapidly and completely metabolized to the parent compound, which is responsible for its antithyroid activity. It is commonly used in treatment of feline hyperthyroidism outside of North America. Carbimazole is a larger molecule than methimazole; 10 mg of carbimazole is equimolar to 6 mg methimazole. To achieve the same effect with carbimazole as with methimazole, approximately twice the dosage of carbimazole would be required. Clinical experience in Europe describes fewer gastrointestinal side effects for this medication likely because this prodrug reduces direct contact of methimazole on gastrointestinal mucosa. Transdermal administration of MMI has also been associated with lower gastrointestinal side effects (Peterson and Becker, 1984; Trepanier, 2007;Frenais et al., 2008, 2009; Daminet, 2014). A controlled release carbimazole tablet was developed, and results of a crossover study demonstrated that the pharmacokinetics of methimazole with a single dose of this prodrug may be similar to those with a single dose methimazole tablet given at an identical molar dose (Longhofer et al., 2010).

Medical Options to Thionamide Drugs

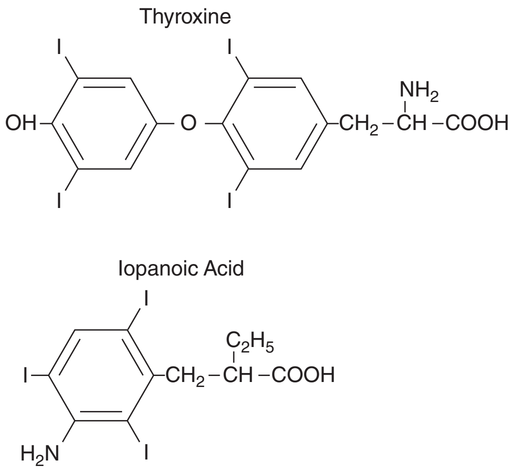
When cats have adverse reactions to the thionamides, if the reaction is gastrointestinal, often a reduction of the dosage or conversion to carbimazole or transdermal therapy will reduce side effects (see Sections Transdermal Methimazole and Carbimazole). However, if the reaction is an allergic one, the antithyroid drug class must be changed if medical therapy is still going to be pursued. PTU and carbimazole, as thionamides with similar structure, would not then be an option; transdermal therapy with methimazole would also not be an option.

Iodinated Radiocontrast Agents

In human medicine, iodinated radiocontrast agents have been employed as adjunctive antithyroid drugs. These agents have several potential mechanisms of action:

1) *Release of iodide, which can transiently suppress thyroid secretion through the Wolff–Chaikoff effect.* This effect seems insignificant in cats as serum T_4 concentrations rarely fall with these agents; however, the effect should be considered if therapy is to precede ^{131}I treatment.
2) *Direct inhibition of both D1 and D2 enzymes reducing the production of more bioactive T_3.* This effect appears to be the most dramatic effect in cats, and efficacy is best monitored by documenting the reduction of serum T_3 into the normal range despite an elevated serum T_4 concentration
3) Inhibition of uptake and/or nuclear receptor binding of T_3. This mechanism has not been documented in cat tissues.

The first such agent used in managing feline hyperthyroidism was the biliary contrast agent, ipodate, which was shown in both experimental hyperthyroidism and in spontaneously hyperthyroid cats to be an alternative medical treatment for patients not tolerating MMI or PTU. In a study of cats in which hyperthyroidism was experimentally induced by the administration of T_4, ipodate significantly reduced the serum T_3 concentrations and was well tolerated by otherwise healthy cats (Chopra et al., 1984; Ferguson et al., 1988; Murray and Peterson, 1997). Ipodate was removed from the market several years ago and has largely been replaced in veterinary use by iopanoic acid. Iopanoic acid contains three iodines and is 67% iodine by weight (Figure 28.10). The dose of 50 mg q 12 h was confirmed in experimentally induced hyperthyroidism and applied in spontaneously hyperthyroid cats. In spontaneously hyperthyroid cats, mean T_3 concentrations fell at all time points and serum T_4 concentrations rose at 1 and 3 months of therapy. The authors concluded that iopanoic acid be useful for acute, but not chronic management of hyperthyroidism (Gallagher and Panciera, 2009, 2011).


Thyroxine

Iopanoic Acid

Figure 28.10 Comparison of structure of radiocontrast agent iopanoic acid to L-thyroxine.

Radioactive Iodine (^{131}I) Therapy

Although only available in specialty referral centers, radioiodine treatment is the most effective and selective cure for toxic goiter in the cat because it selectively destroys the functioning thyroid tissue after being taken up and incorporated into thyroid hormone precursors in the thyroid gland. There is rarely, if ever, damage to the nearby tissue responsible for regulating serum calcium by the secretion of parathyroid hormone and calcitonin. Iodine-131 has a half-life of 8 days and produces both gamma and beta radiation. The beta particles, with a short path length, serve to produce most of the local tissue destruction. Radioiodine is also used in much higher doses in an attempt to ablate thyroid adenocarcinomas in cats and dogs. Following a therapeutic dose (generally 1–5 mCi) of ^{131}I, the serum T_4 and T_3 concentrations will commonly normalize within 1 to 2 weeks. The major disadvantage of radioiodine therapy is that certain radiation safety precautions must be taken. Radioiodine is secreted in saliva, and excreted in urine and feces. As such, handling of the cat's haircoat or waste may result in contamination. Unlike human patients who may receive therapeutic doses on an outpatient basis, radioiodine-treated cats must be hospitalized for periods of time (3 days to 4 weeks), which depend upon the dose administered and the state's radiation safety regulations. Despite these drawbacks, radioiodine therapy is the least invasive cure for bilateral adenomatous goiter, has no hypoparathyroidism or toxicity associated with it, and can be implemented without anesthesia or sedation, an important consideration in the elderly cat with other medical complications (Kintzer, 1994; Kintzer and Peterson, 1991; Meric et al., 1986).

Regarding the efficacy and prognosis for survival following radioiodide therapy, a study of 231 cats being treated with ^{131}I was performed (Slater et al., 2001). With a mean age at diagnosis of 13 years, the treated cats had a median survival time of 25 months. Hypothyroidism was present in five cats (2.2%), and hyperthyroidism was present in one (0.4%). While there was a 33% incidence of renal disease after treatment, it was not clear how many cats might have had this problem at diagnosis (Peterson and Becker, 1995; Peterson, 2006).

Thyroid Imaging

Thyroid imaging is performed using radioactive iodine or $^{99m}TcO_4^-$ (pertechnetate), which is taken up by mechanisms similar to iodide, but is not incorporated into iodothyronines on thyroglobulin. Because of its rapid uptake and increased safety, technetium can be given in higher diagnostic doses and provides a better image than radioiodine. In cats with palpable goiter but normal serum total T_4 and/or free T_4 concentrations, a semiquantitative thyroid scintigraphy is a more sensitive method for confirmation of disease. With this assumption, Daniel and coworkers (2002) used this approach to quantify pertechnetate uptake in 43 cats with hyperthyroidism and eight normal control cats. The 20-minute thyroid : salivary ratio of the most intense lobe correlated best with serum T_4 concentrations, and the authors concluded that this ratio was a valuable predictor of the thyroid status. In a study of over 2000 cases of suspected feline hyperthyroidism, thyroid-to-salivary (T/S) gland ratios >1.5 or thyroid-to-background (T/B)ratios of >6.1 were established as specific diagnostic criteria (Peterson and Broome, 2015). Of this series, 99% had high T/S ratios and 96% had high T/B ratios, 32% had unilateral disease, 63% had bilateral disease, and 4% had multifocal disease. Ectopic lingual or mediastinal disease was established in 4%, and carcinoma was suspected (not proven) in 2% (Kintzer, 1994; Kintzer and Peterson, 1991; Peterson and Broome, 2015).

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29

Glucocorticoids, Mineralocorticoids, and Adrenolytic Drugs

Duncan C. Ferguson and Margarethe Hoenig

Glucocorticoids

Glucocorticoids (GCs) are among the most widely used (and misused) class of drugs in veterinary medicine. In recent years, more has been learned about the actions and adverse effects in a variety of species but, nonetheless, the depth of scientific information on optimal glucocorticoid dosages and dosing intervals in many species is still quite variable as species differences in metabolism and action exist. Therefore, therapeutic protocols are often the product of clinical experience, common sense, and information from human medicine, or from another species. Though the following discussion emphasizes systemic use of glucocorticoids, it should be recognized that local application (ophthalmic, otic, intraarticular, topical, intralesional) also has similar systemic effects.

Management Of Hypoadrenocorticism

Spontaneous glucocorticoid deficiency without concomitant mineralocorticoid deficiency is a relatively rare occurrence in dogs, the species that most commonly suffers from Addison's disease (gluco- and mineralocorticoid deficiency). The underlying cause is suspected to be an immune-mediated destruction of the adrenal cortex, making replacement of the physiological hormones aldosterone and cortisol (in most domestic animals) to be the primary clinical goal. Spontaneous selective glucocorticoid deficiency is rare; however, exogenous glucocorticoids may themselves induce selective atrophy of the glucocorticoid-producing part of the adrenal cortex (zonae fasciculata and reticularis) (Addison, 1855; Ferguson, 1985a; Ferguson et al., 1978; Hoenig and Ferguson, 1991b).

Management of Nonadrenal Disorders: Inflammatory, Allergic, and Autoimmune Disorders

Glucocorticoids are potent antiinflammatory and immunosuppressant agents. The majority of therapeutic applications for these agents fall into these classifications. However, the adverse metabolic effects, as described in Section Toxicity, are difficult to separate pharmacologically from the therapeutic benefits, making glucocorticoids potent, yet potentially dangerous, compounds. The cellular basis for the action of these hormones has recently become clearer and has given rise to new pharmaceutical classes where the antiinflammatory/immunosuppressive and metabolic effects, for example, can be dissociated (see Section Newer Preparations) (Schäcke et al., 2007; Barnes, 2010; Hasselgren et al., 2010; Vandevyver et al., 2013).

Review Of Physiology

Biosynthesis of Steroids

The adrenal cortex synthesizes a variety of steroids from cholesterol and releases them into the circulation. Those steroids with effects on intermediary metabolism are termed "glucocorticoids" and are produced mainly in the layers of the adrenal gland called the *zonae fasciculata* and *reticularis*. The steroids with primarily salt-retaining activity are called *mineralocorticoids* and are synthesized in the zona glomerulosa. The adrenal gland is also capable of synthesizing steroids with androgenic and estrogenic activity. The major glucocorticoid in most domestic animals is cortisol, and in most mammals, the most important mineralocorticoid is aldosterone. In some species (e.g., the rat), corticosterone is the major glucocorticoid. It is less firmly bound to protein and therefore metabolized more rapidly. Quantitatively, dehydroepiandrosterone (DHEA) is the major androgen, with part of it being sulfated to DHEA-sulfate. Both DHEA and androstenedione are very weak androgens. A small amount of testosterone is secreted by the adrenal gland and may be of greater importance as an androgen. Little is known about the estrogens secreted by the adrenal gland. However, the adrenal androgens such as testosterone and androstenedione can be converted to estrone in small amounts by nonendocrine tissues (Aron and Tyrrell, 1994; Tyrrell et al., 1994).

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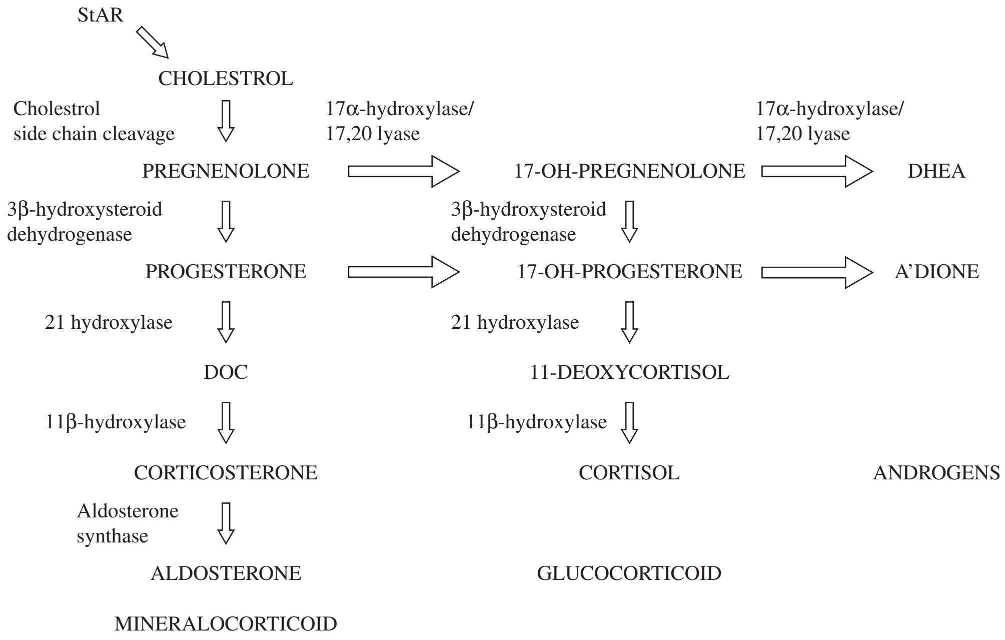

Figure 29.1 Adrenal steroidogenesis. Steroidogenic acute regulatory (StAR) protein mediates uptake of cholesterol into mitochondria within adrenocortical cells. Then aldosterone, cortisol, and adrenal androgens are synthesized through a series of steroidogenic enzymes. A'dione, androstenedione; DHEA, dehydroepiandrosterone; DOC, deoxycorticosterone. Source: Stewart, 2003. Reproduced with permission of Elsevier.

The biochemical pathways involved in adrenal steroidogenesis are shown in Figure 29.1. The initial adrenocorticotropic hormone (ACTH)-dependent rate-limiting step is the transport of intracellular cholesterol from the outer to the inner mitochondrial membrane by steroidogenic acute regulatory (StAR) protein, where it is then converted to pregnenolone by cytochrome P450scc (SCC; side-chain cleavage enzyme). The synthesis of the various adrenal steroids then involves a series of cytochrome P450 enzymes (Table 29.1). SCC and the CYP11B enzymes are localized to the mitochondria and utilize a specific electron shuttle system to hydroxylate steroids. 17α-hydroxylase and 21-hydroxylase are localized in the endoplasmic reticulum. In the cytoplasm, pregnenolone is converted to progesterone by 3β-hydroxysteroid dehydrogenase (3β-HSD), which is the target of the antiadrenal drug trilostane. Progesterone is hydroxylated to 17- hydroxyprogesterone (OHP) by 17α-hydroxylase, and this step is necessary for glucocorticoid synthesis. 21-hydroxylation of either progesterone in the zona glomerulosa or 17-OHP in the zona fasciculata is accomplished by 21-hydroxylase, yielding deoxycorticosterone (DOC) or 11-deoxycortisol, respectively. Cortisol is then synthesized in the mitochondria by the conversion of 11-deoxycortisol to cortisol by the enzyme 11-β-hydroxylase. In the zona glomerulosa, 11β-hydroxylase also converts DOC to corticosterone. Aldosterone synthase also accomplishes this reaction and converts corticosterone to aldosterone through the intermediate 18-OH corticosterone.

Table 29.1 Nomenclature for adrenal steroidogenic enzymes and their genes. Source: Adapted from Stewart, 2003. Reproduced with permission of Elsevier.

Enzyme name	Gene
Cholesterol side-chain cleavage (SCC) (desmolase)	*CYP11A1*
3β-hydroxysteroid dehydrogenase (3β-HSD) (type II isozyme)	*HSD3B2*
17α-hydroxylase/17,20 lyase	*CYP17*
21-Hydroxylase	*CYP21A2*
11β-Hydroxylase	*CYP11B1*
Aldosterone synthase	*CYP11B2*

Adrenal steroids are metabolized in extradrenal tissues as well, and prereceptor enzyme-mediated metabolism of glucocorticoids adds another layer of metabolic control. Cortisol is interconverted with biologically inactive cortisone by the 11-β-hydroxysteroid dehydrogenase system (11-β-HSD). The conversion of inactive 11-ketoglucocorticoids cortisone and 11-dehydrocorticosterone into active 11-β-hydroxyglucocorticoids (cortisol and corticosterone, respectively) is catalyzed by 11-β -HSD1 using NADPH. This enzyme is prominent in metabolically active tissues such as the liver, skeletal muscle, and fat. Likewise, the synthetic glucocorticoid prednisone must be converted by 11-β-HSD1 in the liver for bioactivity (Stewart, 2003). Conversely, 11-β-HSD2 uses NAD^+ to convert cortisol

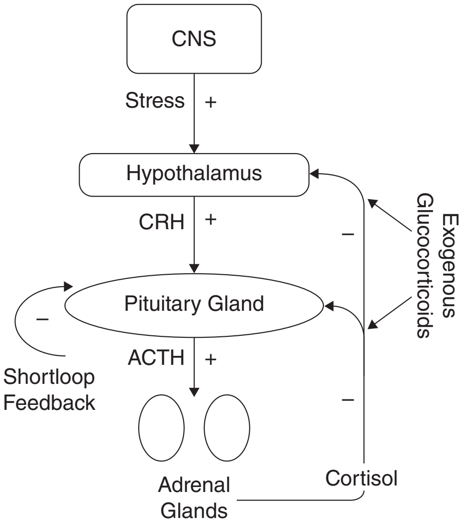

Figure 29.2 Hypothalamic–pituitary–adrenal axis. Exogenous glucocorticoids inhibit the hypothalamic and pituitary function in a negative-feedback fashion. ACTH, through "short-loop" feedback, inhibits its own production. Therefore, recovery of the entire hypothalamic–pituitary–adrenal axis requires withdrawal of glucocorticoid and is not enhanced by the administration of exogenous ACTH. CNS, central nervous system; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotropic hormone; + represents stimulation; – represents inhibition.

to cortisone, essentially inactivating it. In the distal renal tubules, this enzyme inactivates cortisol ensuring that only aldosterone is an agonist at the mineralocorticoid receptors (Atanasov and Odermatt, 2007; Divari et al., 2011; Chapman et al., 2013). The placenta and fetus also express 11-β-HSD2, thereby preventing premature developmental programming that might otherwise be caused by cortisol.

Hypothalamic–Pituitary–Adrenal Axis

Production of ACTH is stimulated by corticotropin-releasing hormone (CRH), which is a hypothalamic hormone (Figure 29.2) (see also Chapter 26); production of ACTH is also influenced by vasopressin (antidiuretic hormone; ADH), particularly during stress. There is also central nervous system (CNS) input into hypothalamic hormone secretion. A "short-loop" feedback system of ACTH on the corticotrophs (ACTH-producing cells) in the pituitary has also been described. In humans, cortisol is secreted in response to pulsatile ACTH release with diurnal variation.

Negative feedback of glucocorticoids on ACTH secretion occurs at both the hypothalamic and pituitary levels via two mechanisms:

1) ”Fast feedback” is sensitive to the rate of change of cortisol levels and likely occurs without interaction with nuclear steroid receptors (see Section Cellular Mechanisms of Negative Feedback).
2) "Slow feedback" is sensitive to the absolute cortisol concentration and is a nuclear receptor–mediated effect, which results in a decrease in ACTH synthesis. The clinical test for spontaneous hyperadrenocorticism, called the dexamethasone suppression test, is mediated by this mechanism of feedback (Keller-Wood, 1990; Tyrrell et al., 1994).

Pharmacological doses of glucocorticoids have a profound effect on endogenous glucocorticoid regulation, suppressing both hypothalamic and pituitary hormone production (Figure 29.2). Cortisol inhibits ACTH secretion and CRH secretion through negative feedback at both hypothalamic and pituitary levels, a point that is important when considering recovery of the hypothalamic–pituitary–adrenal axis (HPAA) from exogenous glucocorticoid administration.

Glucocorticoids with antiinflammatory effects but no effects on the HPAA have not been identified to date. As a result, long-term use of supraphysiological doses may lead to adrenocortical atrophy and decreased adrenal secretory reserve (Chastain et al., 1981; Chastain and Graham, 1979; Hench, 1952; Kemppainen et al., 1982; Moore and Hoenig, 1992).

Cellular mechanisms of negative feedback: Glucocorticoids inhibit pro-opiomelanocortin (POMC) gene transcription (and therefore ACTH synthesis and secretion) in the anterior pituitary and CRH and vasopressin (AVP) mRNA synthesis and secretion in the hypothalamus. This negative feedback is dependent upon the dose, potency, half-life, and duration of administration of the glucocorticoid administered. Although feedback inhibition is principally mediated by the glucocorticoid receptor (GR), there is evidence of fast-loop feedback, which can account for as much as 50% of the negative feedback effect. Glucocorticoids have shown negative effects on spiking of periventricular nucleus (PVN) neurons in the hypothalamus by an effect blocked by a glucocorticoid receptor antagonist. However, GCs also acutely suppress voltage-activated K^+ currents of PVN neurons, and inhibit glutamatergic excitatory postsynaptic currents by PVN neurons within minutes. This effect appears to be due to the release of endocannabinoids, which suppress in a retrograde fashion the presynaptic release of glutamate and γ-amino butyric acid (GABA). Furthermore, GCs induce a rapid facilitatory effect on GABA release by these hypothalamic neurons. There is some evidence that the membrane-bound GRs mediating these rapid effects engage directly or indirectly the Gs-adenylate cyclase-PKA signaling pathway. The combined effect of suppression of excitatory synaptic input with facilitation of inhibition of PVN neurons results in rapid inhibition of PVN output controlling the HPAA (Di et al., 2003; Tasker et al., 2006).

Therapeutic significance: The pattern of glucocorticoid presentation influences gene regulation and tissue responses: pulsatility is an important factor for regulation of the HPAA and of tissue responses to glucocorticoids (Russell and Lightman, 2014). In humans, single daily doses of exogenous glucocorticoids are most commonly recommended to be given in the morning to mimic the adrenal gland's secretory pattern (Tyrrell et al., 1994). Although older reports claimed the existence of a diurnal cortisol variation in dogs and cats, with plasma cortisol level peaking in the morning in dogs and in the evening in cats, carefully designed studies have not confirmed these observations (Kemppainen, 1986). Under basal (nonstressful) conditions, the adrenal gland produces cortisol (hydrocortisone) at about 1 mg/kg body weight daily in most species.

Plasma Binding, Metabolism, and Excretion

In plasma, cortisol is over 90% bound to plasma proteins. The remaining 10% free hormone is the active moiety according to the free-hormone hypothesis. Corticosteroid-binding globulin (CBG), an α_2 globulin synthesized by the liver, binds the majority of circulating hormone under normal circumstances. The remainder is free or loosely bound to albumin and is available to exert its effect on target cells. Cortisol is removed from the circulation by the liver, where it is reduced and conjugated to form water-soluble glucuronides and sulfates, which are excreted into the urine (Aron and Tyrrell, 1994; Grote et al., 1993; Hammond, 1990; Tyrrell et al., 1994).

Molecular Mechanisms of Action

Glucocorticoids have four cellular mechanisms of action: (i) the classical genomic mechanism of action caused by the cytosolic glucocorticoid receptor (cGR); (ii) secondary nongenomic effects also initiated by the cGR; (iii) membrane-bound glucocorticoid receptor (mGR)-mediated nongenomic effects; and (iv) nonspecific, nongenomic effects caused by interactions with cellular membranes (Figure 29.3; Loewenberg et al., 2007). Which of these effects is seen will depend upon the tissue and the dose of glucocorticoids (Table 29.2) (Stahn et al., 2007; Buttgereit, 2004).

Genomic Mechanisms of Action

Genomic mechanisms mediate most effects associated with eventual protein synthesis, such as low-dose physiological replacement therapy and antiinflammatory and immunomodulatory effects; however, it is now clear that some effects, particularly the more rapid ones seen at high glucocorticoid doses, cannot be explained through this mechanism (see Section Nongenomic Effects of Glucocortoids). The genomic mechanisms of glucocorticoid action are cGR-mediated by the cytoplasmic glucocorticoid receptor. The unliganded cGR is a 94-kD protein that exists in the cytoplasm as a multiprotein complex including heat-shock proteins (Hsp), such as Hsp90, Hsp70, Hsp56, and Hsp40. There is also an interaction with immunophilins, (chaperones such as p23 and Src) and several kinases in the mitogen-activated protein kinase (MAPK) signaling system. The glucocorticoid receptor itself consists of three domains: an N-terminal domain containing transactivation functions, a DNA-binding domain with a "zinc-finger" motif, and a glucocorticoid-binding domain. Binding to the cGR ultimately induces (also known as *transactivation*) or inhibits (also known as *transrepression*) the synthesis of regulatory proteins. The genomic effects are generally not observed prior to 30 minutes because of the time necessary for cGR activation/translocation, transcription, and translation. It is estimated that glucocorticoids influence the transcription of ~1% of the entire genome either directly or indirectly through interaction with transcription factors and coactivators (Stahn et al., 2007).

Genomic effects via the cytosolic glucocorticoid receptor (cGR): The cholesterol-like structure and low molecular weight (~300 daltons) allow GCs to pass easily through the cell membrane and bind to the inactive cGR. Conformational changes in the GC/cGR complex result in dissociation from heat shock proteins (HSPs) 70 and 90 followed by migration within 20 minutes to the nucleus. Binding occurs as a homodimer to the glucocorticoid response element (GRE) in association with the activator protein-1 (AP-1) comprised of *c-fos* and *c-jun*. Binding of the GC/cGR to a positive GRE (pGRE) results, for example, in induced synthesis of antiinflammatory proteins (e.g., lipocortin 1, IκB, annexin-1, MAP kinase phosphatase 1, and IL-10), as well as metabolic proteins (e.g., those key to gluconeogenesis) often associated with common side effects. Transcription of genes can be inhibited by GCs via direct interaction between the GR and negative GREs (nGRE), such as those associated with pituitary expression and secretion of pro-opiomelanocortin, the precursor to ACTH, which constitutes the negative feedback system in the pituitary (see Figures 29.2, 29.3) (Loewenberg et al., 2007).

Glucocorticoids also suppress expression of inflammatory genes, including interleukin 1 and 2 (IL-1 and IL-2) via the same mechanism (Loewenberg et al., 2007; Stahn et al., 2007). The repressive effect of GR on nuclear factor-κB (NF-κB) regulation is balanced by the NF-κB negative regulation of GR α–mediated transcription, although the mechanism underlying such antagonism is as yet unclear (Duma et al., 2006). Transcription factors may also be displaced from a positive GRE through direct interaction of the factor and the GR. In addition, GR-mediated transcriptional modulation can be achieved through direct protein–protein interaction with proinflammatory transcription factors, such as activator protein-1 (AP-1),

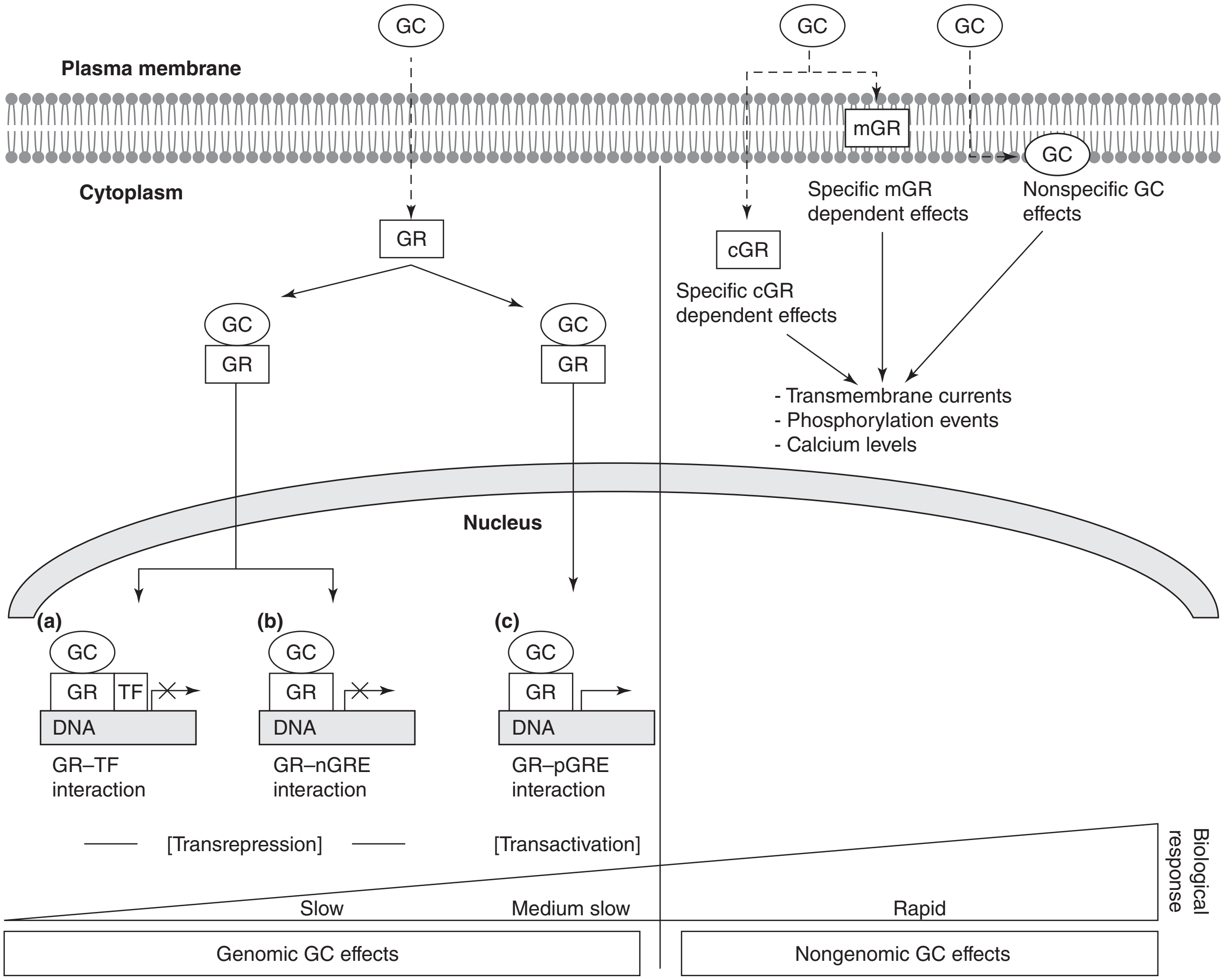

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Figure 29.3 Genomic and nongenomic cellular mechanisms of glucocorticoid (GC) action. GCs diffuse into cells and bind to the cytoplasmic GR, after which the GC–GR complex translocates into the nucleus.
Left side of figure: (a) Ligated GR directly inhibits proinflammatory transcription factors such as AP-1,NF-κB and STAT (b) Ligated GR actively suppresses transcription (transrepression) of inflammatory genes (such as IL-1 and IL-2) through binding to negative GREs (nGREs). GC-induced transrepression is slow because time is required before RNA and protein levels of target genes are fully degraded. (c) Activated GR induces transcription (transactivation) of immunosuppressive genes (e.g., lipocortin-1) via positive GREs (pGREs). Transactivation of genes that encode regulator proteins is less slow (medium slow) compared to transrepression.
Right side of figure: GCs induce rapid effects (occurring within minutes) on transmembrane currents, signal transduction (such as MAPK signaling pathways), second-messenger cascades, or intracellular Ca^{++} mobilization. Nongenomic GC effects are mediated by cytosolic or membrane-bound GRs, or via nonspecific interactions with cell membranes.
In this simplified scheme, no GR–chaperones are depicted.
cGR, cytosolic glucocorticoid receptor; GC, glucocorticoid; GRE, glucocorticoid receptor responsive element; MAPK, mitogen activated protein kinase; mGR, membrane-bound glucocorticoid receptor; TCR, T-cell receptor; TF, transcription factor. Source: Loewenberg et al., 2007. Reproduced with permission of Elsevier.

NF-κB, or signal transducers and activator of transcription (STAT), leading to inhibition of target gene expression (van der Velden, 1998; Loewenberg et al., 2007).

Selective glucocorticoid receptor modulators (SEGRMs) and agonists (SEGRAs): Undesirable effects of GC therapy have generally been ascribed to dimer-mediated transactivation, while beneficial antiinflammatory effects are due to monomer-mediated transrepression of the GR. However, dimer-mediated transactivation is now known to be essential to antiinflammatory activity (Vandevyver et al., 2013). Dissociated GR-ligand agonists (DIGRAs), now preferably called selective glucocorticoid receptor agonist modulators (SEGRAMs, SEGRAs, or SEGRMs), are experimental drugs in development designed to share desirable effects of glucocorticoids with fewer side effects (Schäcke et al., 2007). To the author's knowledge, none have yet made it to veterinary use.

Table 29.2 Current knowledge on the relationship between clinical dosing and cellular actions of glucocorticoids. Source: Adapted from Buttgereit et al., 2004. Reproduced with permission of John Wiley & Sons.

Daily prednisolone dosage (mg/kg)	Therapeutic application	Genomic actions (% receptor saturation)	Nongenomic nonspecific	Nongenomic cGR-mediated
Low (≤0.2)	Replacement or low maintenance Antiinflammatory	+ (<50%)	–	?
Medium (0.2–0.5)	Initial Antinflammatory (chronic disease)	++ (50–100%)	(+)	(+)
High (0.5–1)	Initial Antiinflammatory (subacute) or chronic immunosuppressive	+++ (almost 100%)	+	+
Very high (1–3)	Acute antiinflammatory or life-threatening immunosuppressive	+++ (almost 100%)	+++	++
Pulse or shock therapy (≥3)	Very severe or life-threatening Immunosuppressive or initial lymphocytolytic	++++ (100%)	++++	+++

cGR, cytosolic glucocorticoid receptor.
?, unknown; –, not relevant; (+), perhaps relevant, but of minor importance; +, relevant; ++, relevant to very relevant; +++, very relevant; ++++, most relevant.

Mechanism of action (Figures 29.4): Negative regulation by the GR is associated with inhibition of transcription factor activity (generally proinflammatory) through tethering to such factors. GR homodimer binding to the GRE causes histone acetylation resulting in chromatin remodeling and the association of RNA polymerase II through recruitment of coactivator proteins with acetyltransferase activity, leading to transactivation (e.g., cyclic AMP-responsive element-binding protein). However, when homodimeric GR binds to the negative GRE, either by displacing coactivators or via recruitment of corepressors or histone deacetylases (HDACs), the chromatin structure closes and transcription is inhibited (Schäcke et al., 2007). The GC-induced leucine zipper (*GILZ*) gene has been identified as a downstream mediator of GC antiinflammatory and immunosuppressive effects, and may eventually be a pharmacological target (Ayroldi et al., 2014). Some of these compounds also have shown reduced tendency for insulin antagonism and therefore metabolic side effects (Brandish et al., 2014).

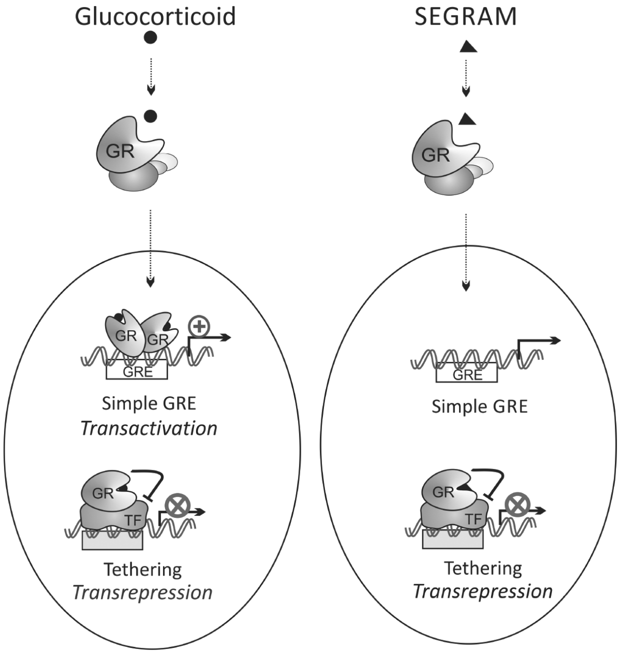


Figure 29.4 Mechanism of transrepression by glucocorticoids and selective glucocorticoid receptor agonists/modulators (SEGRAMs). Simplified overview of the mechanism of action of a classical glucocorticoid versus the mechanism of action of a SEGRAM. GR, glucocorticoid receptor; GRE, glucocorticoid receptor responsive element; TF, transcription factor. Source: https://en.wikipedia.org/wiki/File:SEGRAM_basic_mechanism.tif. Used under CC BY-SA 3.0 http://creativecommons.org/licenses/by-sa/3.0/.

Induction of lipocortin and inhibition of phospholipase A_2: An important mechanism for antiinflammatory and immunosuppressive effects of glucocorticoids is the inhibition of phospholipase A_2 (PLA_2), the enzyme responsible for the release of arachidonic acid from membranes prior to its further metabolism by the cyclooxygenase (COX) and lipoxygenase (LOX) pathways (Figure 29.5). Glucocorticoids enhance the production of a protein called *lipocortin,* which inhibits the enzyme phospholipase A_2 in the cell membrane, thereby inhibiting the formation of prostaglandins, leukotrienes, and PAF (Figure 29.5). Glucocorticoids also may inhibit other phospholipases, such as phospholipase C (Barragry, 1994; Goldfien, 1992; Sorenson et al., 1988). Lipocortin-1, a 37 kDa member of the annexin superfamily of proteins, plays a major regulatory role in systems as diverse as cell growth regulation and differentiation, neutrophil migration, CNS responses to cytokines, neuroendocrine secretion, and neurodegeneration (Flower and Rothwell, 1994).

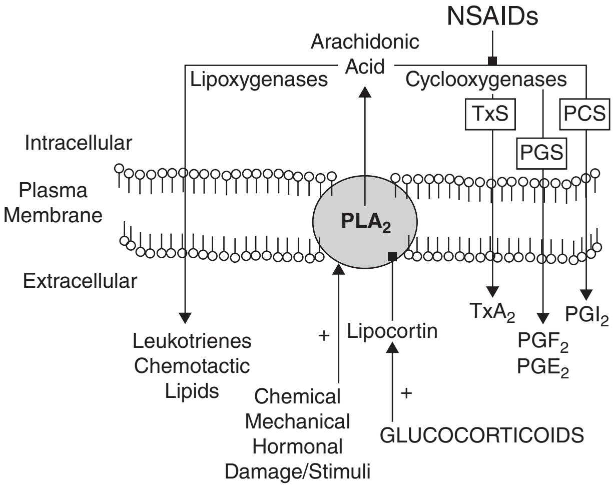


Figure 29.5 Comparison of the effects of glucocorticoids and nonsteroidal antiinflammatory drugs (NSAIDs) on arachidonic acid–derived mediators of inflammation. Glucocorticoids stimulate the production of lipocortin, which inhibits the activity of plasma phospholipase A_2 (PLA_2) and thereby inhibits the release of arachidonic acid and indirectly the production of newly formed inflammatory mediators of the cyclooxygenase pathway as well as the lipoxygenase pathway. The net result is a reduction in production of leukotrienes and chemotactic compounds, as well as less formation of thromboxane A_2 (TxA_2) by thromboxane synthase (TxS), of prostaglandins E_2 and $F_{2\alpha}$ by prostaglandin synthase (PGS), and of prostacyclin (PGI_2) by prostacyclin synthase (PCS).

Steroid receptor isoforms: Glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) form a class of proteins within the nuclear receptor superfamily of receptors including glucocorticoids, mineralocorticoids, estrogens, progesterone, androgen, vitamin D, and retinoic acid. They are widely conserved throughout the species. Glucocorticoid receptors are critical for normal development and differentiation and are essential for life (Duma et al., 2006). For the human GR, there exists a steroid-binding isoform of 777 amino acids, termed α, and a nonsteroid binding β isoform of 742 amino acids, which is identical to the α isoform through the first 727 amino acids (Figure 29.6). Most effects are due to GRα for GR are multiple translation isoforms, and each may undergo different types and degrees of posttranslational modification. Initially, it was thought that GRβ was not physiologically significant but it is now known that it is constitutively expressed in the nucleus and antagonizes the activity of GRα (Ayroldi et al., 2014). GRα is considered the classical GR responsible for most genomic actions, whereas the β isoform has been implicated in the conveyance of glucocorticoid resistance, and possibly a role in autoimmune and inflammatory disorders. It has been suggested that GRβ inhibits GRα transcriptional activity by interference with formation of the coactivator complex.

Much remains to be clarified mechanistically, but it has been suggested that GRβ overexpression may either be neutral, or might lead to anti- or proinflammatory states. There would be pharmacological relevance of GRβ overexpression as an antiinflammatory or to suppress

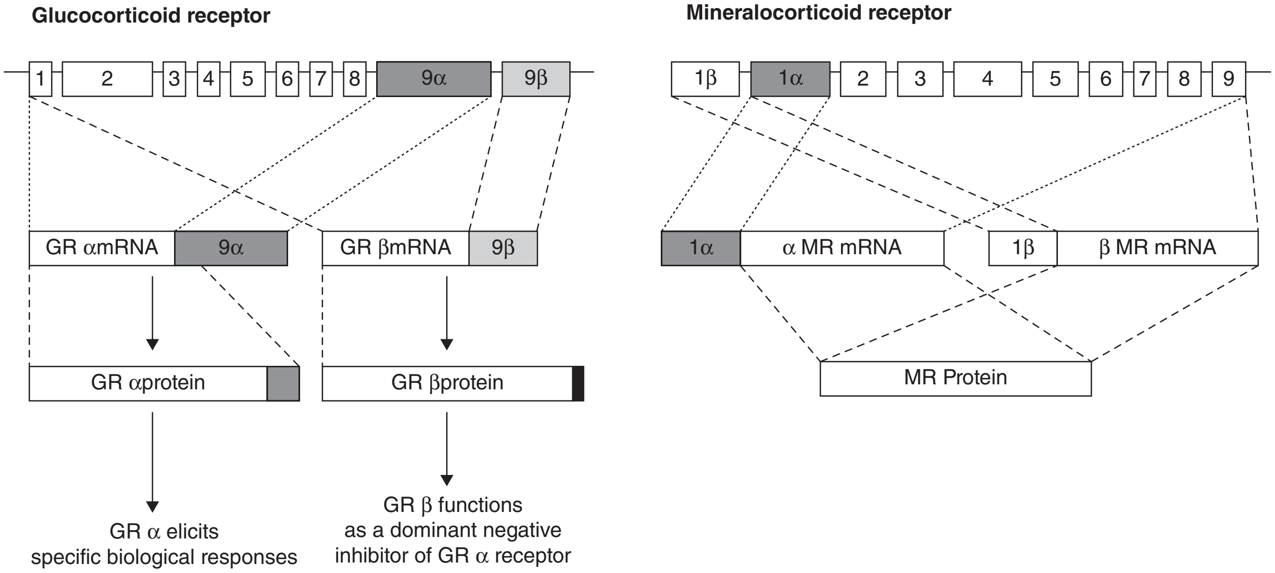


Figure 29.6 Schematic structure of the human genes encoding the glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). Splice variants have been described for both receptors; in the case of the glucocorticoid receptor, there is evidence that the GR β isoform can act as a dominant negative inhibitor of GR α action. mRNA, messenger ribonucleic acid. Source: Stewart, 2003. Reproduced with permission of Elsevier.

metabolic side effects of glucocorticoids. To date, most studies report higher expression of GRβ in autoimmune, inflammatory, or glucocorticoid-resistant states. Neutrophils have a normally high β : α GR ratio, and this may account for their relative glucocorticoid-resistance. However, no study has yet evaluated the effect of acute or chronic glucocorticoid administration on GRβ expression. Nonetheless, proinflammatory cytokines induce an overexpression of GRβ and expression of this GR isoform has been postulated as a possible predictor of therapeutic failure with glucocorticoid therapy (Duma et al., 2006).

Posttranslational modifications: The function of GRs can be subsequently altered by posttranslational modifications, such as phosphorylation, ubiquitination, and SUMOylation. Ultimately, such changes may alter subcellular distribution, transcriptional activity, and protein–protein interactions. Ubiquitination is believed to be a major mechanism by which the degradation rates of GRs are regulated, regulating targeting to proteasome degradation. This is a highly conserved mechanism among eukaryotes. Addition of a small ubiquitin-related modifier-1 (SUMO-1) is called *SUMOylation* and may also impact protein stability and localization. For example, of significance to immunosuppressive or anticancer therapy, the phosphorylation state of the GR may impact glucocorticoid sensitivity during the cell cycle. Glucocorticoid treatment increases phosphorylation during the S phase, but not during the G2 or M phases. As a result, cells synchronized to the S phase were responsive to glucocorticoids, whereas those in G2/M phase were not (Duma et al., 2006).

Nongenomic Effects of Glucocorticoids

In shock and pulse immunosuppressive therapy, very high doses of glucocorticoids are administered. At such doses, cGRs are believed to be saturated (Table 29.2) and effects are unlikely to be limited to receptor-mediated phenomena. At high GC concentrations, nonspecific effects due to direct interaction with the cell membrane lipid may occur (Figure 29.3). These effects are too rapid to be regulated transcriptionally and are termed *nongenomic* but represent several possible mechanisms. Rapid GC effects might occur by interaction of GCs with specific membrane receptors. For example, a plasma-membrane form of GR has been shown in B cells and peripheral blood mononuclear cells. GCs may also act on the plasma membrane by nonspecific physicochemical mechanisms, particularly when at high concentrations. Through such mechanisms, they may inhibit Na^+ and Ca^{++} plasma membrane transport or increase H^+ leak of the mitochondria. The former mechanism in lymphocytes has been implicated as part of the rapid immunosuppression induced by glucocorticoids. The effects of GCs on ion fluxes and vascular permeability are independent of protein synthesis (Muller and Rankawitz, 1991; Reul et al., 1990). In fact, the rapid effect of GCs at inhibition of allergic conditions has been shown to be caused by a reduction in intracellular calcium (Zhou et al., 2008). Nongenomic effects of glucocorticoids have also been described on different cellular processes, such as actin polymerization, neuronal membrane conductance, and other signal transduction mechanisms. For example, short-term glucocorticoid treatment reduced antigen-induced phosphorylation of mitogen-activated protein (MAP)–extracellular signal-regulated kinase (ERK, MEK), as well as reduced phospholipase A_2 (PLA_2) (Song and Buttgereit, 2006).

Specific membrane-associated receptors for glucocorticoids may be involved in the rapid effects of these agents in shock conditions (Gametchu et al., 1991; Grote et al., 1993; Liposits and Bohn, 1993). Glucocorticoids as cholesterol-like compounds, may change membrane fluidity, thereby regulating plasma membrane $GABA_A$, EGF, insulin-like growth factor-I (IGF-I), and dopamine receptors. Receptor-mediated insertion of steroid hormones into DNA may also take place, with the steroid acting as a transcription factor. These diverse modes of action provide for integrated rapid and/or prolonged effects to address the physiological needs of the individual (Brann et al., 1995).

Glucocorticoids can induce the rapid phosphorylation and membrane translocation of annexin-1, an important antiinflammatory protein. They also have been shown to activate endothelial nitric oxide synthase (eNOS) via actions on phosphatidylinositol 3-kinase and protein kinase B, resulting in vasodilatation associated with cardio protective effects of glucocorticoids. Neuroprotection following high-dose glucocorticoids may also be associated with a nongenomic effect, again possibly by eNOS activation and increased cerebral blood flow (Song and Buttgereit, 2006).

Membrane-bound states of the cytosolic glucocorticoid and mineralocorticoid receptors have also been described. Aldosterone receptors on plasma membranes of lymphocytes and smooth muscle cells appear to trigger changes in Ca^{++}, IP3, cAMP, and protein kinase C. Supporting clinical significance, the number of plasma membrane-bound GRs correlates with the ability of GCs to induce lymphocyte death. Further work is necessary to clarify the functional role and cellular mechanisms associated with membrane-bound GR. Current thinking is that there is only one cytosolic GR, which may be either unliganded and membrane-associated, or liganded and act as a "classical" nuclear transcription factor. In future pharmaceutical development, glucocorticoids with preference for membrane-bound GRs may have enhanced immunosuppressive activity with fewer side effects (Buttgereit et al., 2004; Song and Buttgereit, 2006; Loewenberg et al., 2007).

Physiological Effects of Glucocorticoids

A principal role of glucocorticoids is the maintenance of fluid homeostasis by regulation of volume and composition of body fluids and by being permissive for essential cellular metabolism. Without glucocorticoids, an animal cannot survive a stressful incident. The glucocorticoids have widespread effects because they influence the function of most cells in the body. Glucocorticoids in physiological quantities are essential for the dilution of the renal filtrate into the hyposthenuric range. Other physiological roles of glucocorticoids are to increase gluconeogenesis, decrease protein synthesis, and increase lipolysis with the release of glycerol and free fatty acids (an insulin-antagonistic effect). Although many of the effects are dose related, glucocorticoids may also act in a permissive manner to optimize certain cellular reactions such as the gluconeogenesis stimulated by glucagon and catecholamines. The physiological effects of glucocorticoids in the fed state are not very significant; however, during fasting, glucocorticoids contribute to the maintenance of glucose concentrations by increasing the release of glucose by the liver and increasing gluconeogenesis and glycogen deposition by stimulating glycogen synthase. The effect on muscle is catabolic because glucose uptake decreases and amino acid release (gluconeogenesis) increases.

Glucocorticoids stimulate adipocyte differentiation, promoting adipogenesis through activation of key adipocyte differentiation genes such as those coding for lipoprotein lipase, glycerol-3-phosphate dehydrogenase, and leptin. Glucocorticoids also are permissive for activity of the hormone-sensitive lipase (HSL), which is responsible for mobilization of free fatty acids from adipose stores. Lipolysis is stimulated, therefore, when insulin is lacking or being greatly antagonized; ketogenesis may result (Aron and Tyrrell, 1994; Ferguson, 1985a; Goldfien, 1992; Haynes, 1990; Melby, 1974; Wilcke and Davis, 1982).

Glucocorticoids also maintain microcirculation, normal vascular permeability, and stability of lysosomal membranes, and suppress inflammatory reactions, although these functions are more commonly associated with the pharmacological effects of glucocorticoids (Aron and Tyrrell, 1994; Tyrrell et al., 1994).

Pharmacological Effects

Growth and Development

Glucocorticoids play a physiological role in the development of pulmonary surfactant in the near-term fetus, allowing an adaptation to air breathing. GCs stimulate lung maturation through the synthesis of surfactant proteins. GR knockout mice do not survive because of lung atelectasis. Prematurity with delayed development of the adrenal axis in foals has been suspected as a cause of neonatal respiratory distress syndrome. Although glucocorticoids stimulate growth hormone (GH) gene transcription in physiological quantities, glucocorticoids in excess inhibit linear skeletal growth, via catabolic effects on connective tissue and muscle and inhibition of IFG-1 effects. GCs also stimulate the adrenal medullary enzyme converting norepinephrine to epinephrine (Aron and Tyrrell, 1994; Tyrrell et al., 1994; Stewart, 2003).

Energy Metabolism

Glucocorticoids have an antagonistic effect to that of insulin, leading to increased glucose production from amino acids (gluconeogenesis) and reduced incorporation of amino acids into protein. As stated above, glucocorticoids enhance lipolysis; however, glucocorticoid excess (pharmacological amounts) or spontaneous hyperadrenocorticism may result in redistribution of fat because glucocorticoids stimulate appetite, thereby stimulating hyperinsulinemia, which results in lipogenesis. As a result, diabetes mellitus may result from prolonged glucocorticoid use at high dosages in animals with diminished insulin secretory capacity (prediabetics). Muscle wasting and weakness are not uncommon with glucocorticoid excess; although glucocorticoids stimulate protein and RNA synthesis in the liver, they have catabolic effects in lymphoid and connective tissue, muscle, fat, and skin. While not usually a recognizable clinical problem in domestic animals, osteoporosis may result in people with Cushing's syndrome or on chronic glucocorticoid administration. The problem likely is most significant in areas of healing bone; glucocorticoids directly inhibit bone formation by inhibiting osteoblast proliferation and the synthesis of bone matrix while stimulating osteoclast activity. In addition, glucocorticoids potentiate the action of parathyroid hormone (PTH) and 1,24-dihydroxycholecalciferol ($1,25(OH)_2$-D_3) and inhibit the gut absorption of calcium, an effect that can be used to advantage in hypercalcemic states. However, in the young animal, the catabolic effects of excessive amounts of glucocorticoid reduce growth. In children, this reduced growth is not prevented by growth hormone (Aron and Tyrrell, 1994; Tyrrell et al., 1994). In adult dogs, 2 mg/kg prednisone for 30 days reduced bone mineral density by 14% as measured by helical computer tomography (Costa et al., 2010).

Water and Electrolyte Balance

Glucocorticoid use invariably leads to polyuria and polydipsia via inhibition of ADH release and action, as well as alteration of the animal's psyche, resulting in increased water intake. No glucocorticoid given in large doses is completely devoid of mineralocorticoid (salt-retaining and K^+-losing) activity; therefore, excessive use may precipitate or exacerbate hypertension and induce hypokalemia. In part by increasing extracellular fluid volume, glucocorticoids increase the glomerular filtration rate and are required in physiological amounts for maximal dilution of urine (Ferguson, 1985a; Melby, 1974; Nakamoto et al., 1992; Smets et al., 2012).

Immune and Hematological Effects

Often the desired result of a therapeutic application, antiinflammatory effects of glucocorticoids are primarily seen at pharmacological doses. Glucocorticoids result in alterations in the concentration, distribution, and function of peripheral leukocytes and in inhibition of phospholipase A_2 activity in the plasma membranes of these cells. Glucocorticoids act indirectly by inducing lipocortin synthesis, which in turn inhibits arachidonic acid release from membrane-bound stores, and also by inducing transforming growth factor-β (TGF-β) expression, which subsequently blocks cytokine synthesis and T-cell activation. In addition to contributing to maintenance of the microcirculation and cell membrane integrity, glucocorticoids interfere with progressive dissolution and disruption of connective tissue and cells, possibly by stabilizing lysosomal membranes (Aron and Tyrrell, 1994; Aucoin, 1982; Barragry, 1994). Although lysosomal stabilization by glucocorticoids has been demonstrated experimentally, it is hard to know what benefit these effects have in clinical situations. Glucocorticoids also decrease formation of induced histamine (histamine produced locally by cells during injury), the action of which is not effectively blocked by standard doses of antihistamines. They also antagonize toxins and kinins, reducing the resultant inflammation. It is important to realize, however, that most of their effects are *nonspecific;* that is, they have profound metabolic effects regardless of the initial insult.

Glucocorticoids are used to advantage to suppress both the number of cells and the actions of the immune system. The suppressive effects on cell-mediated immunity predominate over those on humoral immunity. Antibody production is generally unaffected by moderate dosages of glucocorticoids and is inhibited only at high dosages and with long-term therapy. They cause lymphopenia and eosinopenia, an effect secondary to cell redistribution and/or lysis, and lead to increased vascular demargination of neutrophils from the vascular bed to lymphoid tissue. Glucocorticoids inhibit virus-induced interferon synthesis and diminish the functional capacity of monocytes, macrophages, and eosinophils through inhibition of the formation of ILs such as IL-1 (macrophages), IL-2 (lymphocytes), IL-3, and IL-6 and other chemotactic factors. Glucocorticoids can induce apoptosis on normal lymphoid cells and play a key role in the physiology of thymic selection. They inhibit monocyte differentiation into macrophages and macrophage phagocytosis and cytotoxic activity (Barragry, 1994; Ehrich et al., 1992; McDonald and Langston, 1994; Melby, 1974; Tyrrell et al., 1994; Buttgereit et al., 2004).

In recent years, greater understanding of the mechanisms of GC inhibition of cell-mediated immunity has developed, and the GC-induced leucine zipper (*GILZ*) gene has emerged as a critical mediator. In response to GCs, GILZ up-regulates and inhibits T cells through inhibition of anti-CD3-induced activation and apoptosis. GILZ also interacts with and inhibits NF-κB. Other GILZ targets have been identified, including AP-1, Raf-1, and Ras, and each has been implicated in the effects of GCs. GILZ silencing diminishes the antiproliferative activity of dexamethasone and reduces inhibition of cytokine-induced COX-2 expression (Ayroldi and Riccardi, 2009; Ayroldi et al., 2014). In addition to the GILZ-mediated effects, GCs appear to up-regulate repair-phase cytokines like TGF-β and platelet-derived growth factor (PDGF), possibly explaining the suppressive effect on healing and fibrosis (Pallardy and Biola, 1998; Buttgereit et al., 2004).

In the clinical setting, GCs are used for their ability to induce apoptosis of malignant lymphoid cells. The mechanisms of apoptosis induced by glucocorticoids fall roughly in two categories, depending on the type of lymphocytes: induction of "death genes" such as IκB and *c-jun* or repression of survival factors such as AP-1 and *c-myc.* By inhibiting the production of Th1 cytokines, glucocorticoids may enhance Th2 cell activity and generate a long-lasting state of tolerance.

Cardiorespiratory Effects

In addition to indirect effects on electrolyte metabolism, glucocorticoids have direct positive chronotropic and inotropic actions on the heart. They appear to block the increased permeability of capillaries induced by acute inflammation, reducing transport of protein into damaged areas and maintaining microcirculation. Because glucocorticoids are necessary for maximal catecholamine sensitivity, they contribute to maintenance of vascular tone (Ferguson et al., 1978; Nakamoto et al., 1992). In shock, production of vasoactive products of lipid peroxidation (arachidonic acid cascade), such as the vasoconstrictor thromboxane A_2, may be decreased by glucocorticoids, but probably only in the early stage of cell disruption. Glucocorticoids cause vasoconstriction when applied directly to vessels. They decrease capillary permeability by inhibiting the activity of kinins and bacterial endotoxins and by reducing the amount of histamine released by basophils.

Glucocorticoids may induce hypertension in animals and humans through the following mechanisms: (i) activation of the renin–angiotensin system due to an increase in plasma renin substrate; (ii) reduced activity of the hypotensive kallikrein–kinin system, prostaglandins (PGs), and the endothelium-derived relaxing factor, nitric oxide (NO); and (iii) increased pressor responses to angiotensin II and norepinephrine. Furthermore, the number of angiotensin II type 1 receptors of vascular smooth muscle cells is significantly increased by glucocorticoids (Saruta, 1996).

Glucocorticoids increase the number and affinity of β-adrenergic receptors. Glucocorticoids prevent receptor down-regulation and therefore tachyphylaxis, resulting in potentiation of the effects of β-adrenergic agonists on bronchial smooth muscle, an important effect in the asthmatic patient (Sprung et al., 1984; Tyrrell et al., 1994; Wilcke and Davis, 1982).

CNS Effects

Although rarely described in domestic animals, glucocorticoids (or lack of them) have marked effects on the psyche, resulting in a form of mental, as well as physical, dependence (Ferguson, 1985a; Metz et al., 1982). It is known that pretreatment of neonatal rats with dexamethasone provides protection against hypoxic–ischemic brain damage. This effect is likely mediated via glucocorticoid receptors, because glucocorticoid receptor antagonist RU38486 reverses the benefit. The neuroprotection also appears to be related to alterations in cerebral metabolism. Glucose utilization is reduced prior to hypoxia–ischemia and is better maintained following dexamethasone. High-energy phosphates in the brain are higher in dexamethasone-treated animals. Thus, glucocorticoids may provide their protection against hypoxic–ischemic damage by decreasing basal metabolic energy requirements and/or increasing the availability or efficiency of use of energy substrates (Tuor, 1997).

Endocrine Effects

Glucocorticoids, in addition to being diabetogenic, also have marked effects on hypothalamic and pituitary function. ACTH, β-lipotropin, thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and growth hormone (GH) synthesis and secretion are all suppressed; however, β-endorphin levels are unaffected. Glucocorticoids, even at "physiological" dosages (0.22 mg/kg prednisolone once daily orally in the dog), result in HPAA suppression, as indicated by reduction in the ACTH-stimulated cortisol concentration increment and by reduction of the ratio of the zona fasciculata and reticularis to zona glomerulosa in the adrenal gland. Antiinflammatory dosages of prednisolone (0.5 mg/kg q 12 h orally) resulted in adrenal suppression within 2 weeks of therapy (Chastain and Graham, 1979). In another study in dogs, 1 month of the same dosage of prednisolone orally resulted in profound suppression of endogenous plasma ACTH and cortisol concentrations, CRH-stimulated ACTH release, and ACTH-stimulated cortisol release. However, following withdrawal of the prednisolone, the HPAA returned to normal within 2 weeks (Moore and Hoenig, 1992). A similar ability to recover from exogenous glucocorticoids was seen in the cat. A dosage of 2 mg/kg q 12 h of methylprednisolone given orally for 7 days resulted in suppression of ACTH-stimulated cortisol and CRH-stimulated ACTH, but these changes completely reversed by 7 days after withdrawal of the exogenous glucocorticoid (Crager et al., 1994). Higher dosages and long-acting preparations (dexamethasone, triamcinolone, depot products) may result in more pronounced HPAA suppression (Kemppainen and Sartin, 1984; Kemppainen, 1986; Kemppainen et al., 1982). Even hydrocortisone at 10 mg/kg twice a day for 4 months led to ultrasonographically evident adrenal atrophy that was reversible after 1 month of withdrawal (Pey et al., 2012).

The effect of glucocorticoids on glucose metabolism is time and dose dependent; insulin and glucose concentrations, or glucose tolerance, were not significantly altered by the administration for 28 days of an antiinflammatory dosage of oral prednisone (Moore and Hoenig, 1993). However, daily administration of high dosages of dexamethasone and growth hormone is a reliable model for induction of diabetes mellitus in the cat (Hoenig et al., 2000). There also have been case reports of diabetes mellitus induced in dogs after administration of corticosteroids and methylprednisolone pulse therapy (Jeffers et al., 1991).

Pharmacological doses of glucocorticoids generally reduce serum thyroid hormone concentrations, presumably through suppression of pituitary TSH. These effects have been well documented in the dog, are not as significant in the cat, and are not well studied in other domestic species (Ferguson and Peterson, 1992; Kaptein et al., 1992; Moore et al., 1993). The metabolic consequences of these lowered concentrations of thyroid hormones are not known in the dog; however, a state of hypothyroidism is not believed to be the result (Jennings and Ferguson, 1984).

Gastrointestinal Effects

Physiological glucocorticoid responses contribute to the maintenance of the gastric mucosal integrity following ulcerogenic stimuli. The role of glucocorticoids in gastroprotection becomes especially important where there is deficiency of PGs or NO or desensitization of intestinal sensory neurons (CSN). In rats with normal corticosterone, these insults do not result in gastric mucosal disruption, but do in adrenalectomized rats (Filaretova et al., 2007). These findings may explain the observation of bloody diarrhea in dogs with glucocorticoid deficiency and/or Addison's disease.

However, pharmacological doses of glucocorticoids stimulate excessive production of acid and pepsin in the stomach and may cause peptic ulcer. In an experimental canine fundic pouch model of acid secretion, the effect of prednisolone (2 mg/kg per day given parenterally for three doses, 2 weeks or 12 weeks) on gastric mucosal H^+ permeability was studied. Acute administration (<2 weeks) had no effect. Chronic administration (12 weeks) increased slightly the basal H^+ secretion rate, and increased mucosal permeability by 50% when simultaneously administered with aspirin (Chung et al., 1978). Similarly, 0.25 mg/kg SC q 12 h for 3 days induced endoscopically evident gastric lesions in dogs (Boston et al., 2003). Glucocorticoid lesions are exacerbated by simultaneous administration of nonsteroidal antiinflammatory drugs (NSAIDs) (Dow et al., 1990; Narita et al., 2007). In rats, dexamethasone-induced ulceration has led to alterations in the phospholipid fatty acid profile (increased linoleic acid and decrease in other polyunsaturated fatty acids, PUFAs). PUFA supplementation or the administration of an H_2 receptor antagonist reduced ulcerization with near normalization of changes in the phospholipid fatty acid profile (Manjari and Das, 2000).

Prednisolone and dexamethasone, studied in canine colon cell culture, had no effect on the intestinal cell tight junction barrier function, but did prevent the increase in permeability induced by TNF-α. Specifically, glucocorticoids, acting through nuclear receptors, inhibited the TNF-α–induced increase in myosin light chain kinase protein expression, which was shown to mediate increased intestinal tight junction permeability (Boivin et al., 2007).

Glucocorticoids facilitate fat absorption and appear to antagonize the effect of vitamin D on calcium absorption. Therefore, glucocorticoids are employed in chronic hypercalcemic states in an attempt to inhibit gastrointestinal calcium absorption (Aron and Tyrrell, 1994; Tyrrell et al., 1994). Glucocorticoid therapy may cause calcinosis cutis in dogs, but no specific dose or set of factors has been identified to consistently cause it (Doerr et al., 2013).

Chemistry

Source

Although the natural corticosteroids can be obtained from animal adrenal glands, they are usually synthesized from cholic acid or steroid sapogenins found in plants of the Liliaceae and Dioscoreaceae families. Further modifications of these steroids have led to the marketing of a large group of synthetic steroids with special characteristics that are pharmacologically and therapeutically important (Table 29.3 and Figures 29.7 and 29.8).

Structure–Activity Relationships

The actions of the synthetic steroids are similar to those of cortisol (see Section Pharmacological Effects). They bind to the specific intracellular receptor proteins and produce the same effects but have different ratios of glucocorticoid-to-mineralocorticoid potency (see Table 29.3).

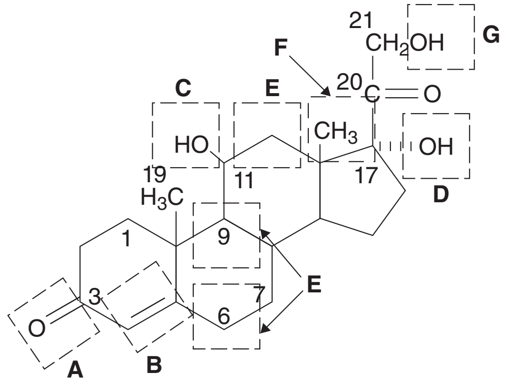
Table 29.3 Characteristics of various glucocorticoid bases

	Potency			
Drug	**Glucocorticoid**[a]	**Mineralocorticoid**	**HPAA suppression**	**Alternate-day therapy possible?**
Short-acting (duration of action: <24 hours)				
Hydrocortisone	1	++	+	No (too short)
Cortisone	0.8	++	+	Yes (not ideal)
Prednisone	4	+	+	Yes
Prednisolone	4	+	+	Yes
Methylprednisolone	5	+	+	Yes
Intermediate-acting (duration of action: 24–48 hours)				
Triamcinolone	5	0	++	No
Long-acting (duration of action: <48 hours)				
Flumethasone	15	0	+++	No
Dexamethasone	30	0	+++	No
Betamethasone	30	0	+++	No

Effective antiinflammatory time equals the HPAA suppression time in most cases. However, the therapeutic success and fewer side effects with alternate-day therapy stem from the fact that some preparations have slightly longer antiinflammatory or immunosuppressive action than their action to suppress the HPAA.

[a] Compared with hydrocortisone on a mg-for-mg basis.

HPAA, hypothalamic–pituitary–adrenal axis.



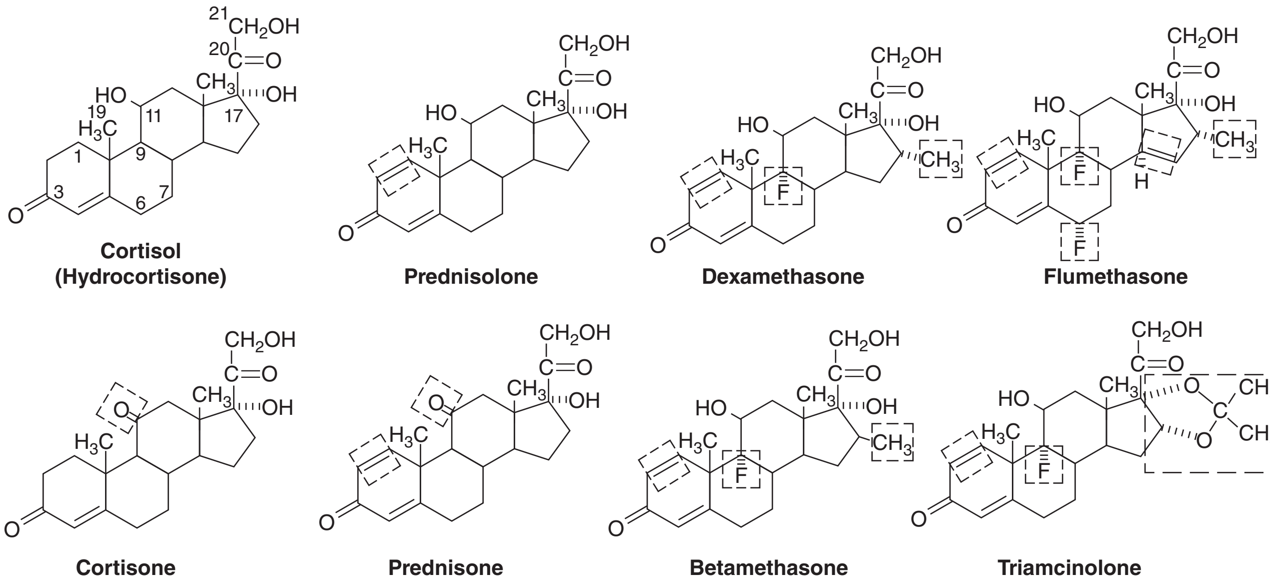
Cortisol

Figure 29.7 Structure–activity relationships of glucocorticoids. Shown on the structure of the compound cortisol are the important structural sites determining the activity of a glucocorticoid base. *A:* 3 keto group essential for glucocorticoid activity; *B:* 4,5 double bond essential for glucocorticoid activity; *C:* 11 hydroxyl essential for optimal glucocorticoid activity; *D:* 17 α hydroxyl is important for glucocorticoid activity; *E:* 16 methylation or fluorination reduces mineralocorticoid activity considerably and increases glucocorticoid activity; *F:* 20 keto group is important for glucocorticoid activity; *G:* 21 hydroxyl is essential for mineralocorticoid activity and is the site of esterification.

Steroid Base

Figures 29.7 and 29.8 show the steroid base, or carbon skeleton, of glucocorticoids. The structure of the base determines the *antiinflammatory (glucocorticoid) potency, mineralocorticoid potency,* and the *duration of action* once at the site of action.

Alterations in the steroid base structure influence its affinity for glucocorticoid and mineralocorticoid receptors, as well as its protein-binding avidity, side-chain stability, rate of reduction, and metabolic products. Certain structures on the steroid base are essential for glucocorticoid activity (Figures 29.7 and 29.8). An 11-ketol is essential for glucocorticoid activity, and compounds like cortisone and prednisone must first be reduced in the liver from the 11-carbon ketone to the ketol before full activity is seen. The 1,2 double bond provides a fourfold increase in glucocorticoid activity. The C-3 and C-20 ketone groups are also essential for glucocorticoid activity. The addition of 16-α-methyl 9-α-fluoro groups results in compounds with enhanced antiinflammatory activity. Further substitution at the 17 ester position results in a new group of extremely potent steroids (e.g., beclomethasone and betamethasone) that are effective when applied topically for skin diseases and by inhalation for treating asthma. Modifications of the glucocorticoid molecular structure also alter the tendency for the molecule to bind with CBG in the plasma. An increase in binding to CBG results in a lower tendency for the hormone to be metabolized. Halogenation at the 9 position, unsaturation of the 1,2 bond, and methylation at the 2 or 16 position will prolong the half-life by more than 50–70%. The 11-hydroxyl group also appears to inhibit destruction, since the half-life of 11-deoxycortisol is half that of cortisol. In some cases, the agent administered is a prodrug; prednisone is rapidly reduced to prednisolone, and cortisone is rapidly converted to cortisol by the liver. The synthetic corticosteroids for oral use are in most



Cortisol (Hydrocortisone)

Prednisolone

Dexamethasone

Flumethasone

Cortisone

Prednisone

Betamethasone

Triamcinolone (acetonide)

Figure 29.8 Structures of common glucocorticoids used in veterinary medicine. The key elements of structure differing from cortisol are identified by the outlined areas.

cases rapidly and completely absorbed when given by mouth.

Ester

Esterification of the alcohol at C-21 serves a number of potential purposes. The ester moiety determines to a significant extent the water/lipid solubility ratio and also influences the duration of action of the base compound's release from subcutaneous or intramuscular sites. Tissue esterases cleave the ester, resulting in free base, which then is distributed via the circulation to tissue sites of action. Routes of administration and duration of release are given for the following esters:

1) phosphate and hemisuccinate: IV or IM, rapid action and metabolism;
2) acetate, diacetate, tebutate: subcutaneous or IM depots, 2–14 days;
3) acetonide: subcutaneous or IM depots, poorly H_2O soluble;
4) pivalate: subcutaneous or IM depots; weeks to months.

The ability of a formulation to suppress the HPAA is determined by the *dosage,* the *potency of the base,* and the *duration of action of the formulation* (base + ester).

Toxicity

The main limiting factors for glucocorticoid administration are the global toxic effects of these agents. Table 29.4 lists most of the reported side effects as described in the dog, the species in which glucocorticoids are most widely used. Of course, there is variability from species to species. The following discussion highlights the basis for some of these effects.

Metabolic/Endocrine Effects

The endocrine manifestations of glucocorticoid administration can be severe. Iatrogenic Cushing's syndrome may develop with adrenal insufficiency on withdrawal of the medication. The gluconeogenic effects may unmask or exacerbate diabetes, and polyuric and polydipsic states and may induce hypertension.

CNS Effects

In animals, the effects on mental status are difficult to assess or compare with those in humans; however, glucocorticoids likely induce a state of well being. Neurologically, there is also evidence that glucocorticoids may decrease the threshold for seizures. Lethargy and panting occasionally develop in dogs and cats. Rapid withdrawal of glucocorticoids can induce depression and irritability (Ferguson, 1985a, 1985b; McDonald and Langston, 1994).

Table 29.4 Reported side effects of glucocorticoid treatment (with emphasis on dogs). Source: Kemppainen, 1986. Reproduced with permission of Elsevier.

Blood and blood chemistry
Increases in:
  Neutrophils
  Erythrocytes
  Monocytes
  Platelets
  Alkaline phosphatase[a]
  Cholesterol
  Glucose
  Alanine aminotransferase
Decreases in:
  Eosinophils[a]
  Lymphocytes
  Blood urea nitrogen

Central nervous system
Behavioral and mood changes (depression, increased irritability)
Lethargy
Panting

Endocrine
Iatrogenic Cushing's disease, HPAA suppression[a], secondary adrenocortical insufficiency
Reduced thyroid hormone (T_4 and T_3) levels
Reduced gonadotropin and sex steroid levels
Anestrus, testicular atrophy, reduced libido
Elevated insulin levels, carbohydrate intolerance
Reduced vitamin D levels
Elevated parathyroid hormone levels

Gastrointestinal
Polyphagia
Anorexia (rare)
Diarrhea (may be bloody)
Increased gastric acid secretion
Hepatomegaly
Hepatopathy
Pancreatitis
Colonic perforation

Renal
Polyuria with secondary polydipsia[a]
Increased urinary calcium excretion

Musculoskeletal
Muscle atrophy
Weakness, exercise intolerance
Myotonia (rare)
Osteoporosis

Skin
Calcinosis cutis
Thin skin
Bilateral hair loss
Increased bruising

Other
Increased risk of infection
Enhanced spread of infection
Poor wound healing
Redistribution of body fat
Reduced growth

[a] Relatively common finding in dogs.
HPAA, hypothalamic–pituitary–adrenal axis; T_3, triiodothyronine; T_4, thyroxine.

Lending to the understanding of their potential protective effects at high doses in central nervous system injury, glucocorticoids have been shown to protect neurons from apoptosis by a mechanism involving the cyclin-dependent kinase inhibitor $p21_{Waf1/cip1}$, an effect reversed by receptor antagonists as well as inhibitors of PI3- and Akt-kinase (Harms et al., 2007).

Gastrointestinal and Hepatic Effects

The gastrointestinal and hepatic effects of glucocorticoids are among the most limiting regarding chronic administration. Glucocorticoids clearly induce a form of micronodular cirrhosis and stimulate the steroid-specific isozyme of alkaline phosphatase. In addition to hepatopathy and hepatomegaly, glucocorticoid excess may result in increased gastric acid and gastric ulceration, but more commonly high doses of potent glucocorticoids cause colonic perforation in dogs. Of course, glucocorticoids may stimulate appetite, an effect occasionally used to advantage in some therapeutic situations. Rarely, the animal will respond with anorexia. It has been postulated that glucocorticoids may also cause pancreatitis.

Musculoskeletal Effects

Chronic glucocorticoid administration induces excessive catabolism and muscle atrophy. Animals may become clinically weak and be unable to exercise optimally. Bone growth may be inhibited and antagonism of vitamin D activity may result in osteoporosis with chronic administration of glucocorticoids.

Dermatological Effects

Glucocorticoids reduce collagen synthesis and thereby reduce the rate of wound healing. The skin becomes thin and more easily stretched and bruised due to increased capillary fragility. Dogs on large dosages of glucocorticoids often develop bilateral symmetrical alopecia, known as a "cushingoid" appearance. Cats tend to be less susceptible to these effects. Occasionally, exogenous glucocorticoid administration will induce calcium deposition in the dystrophic epidermis; however, the incidence in dogs appears to be less than with spontaneous hyperadrenocorticism (Scott, 1982).

Immunological Effects

Although immune suppression is often the desired therapeutic effect of glucocorticoids, these agents are notorious for exacerbation of clinical or latent infectious disease processes. Animals on chronic glucocorticoid therapy have a higher incidence of bacterial infections; in one study, 75% of dogs on glucocorticoids for allergic skin disease had clinical or subclinical urinary tract infections. Of course, by inhibiting the cell-mediated immune response to an infection, glucocorticoids slow the function of immune cells that help contain an infection (Aucoin, 1982; Fauci, 1976; Ferguson, 1985a, 1985b; Siegel, 1985).

Reproductive Effects

High doses of glucocorticoids induce parturition during the latter part of pregnancy in ruminants and horses (Barragry, 1994). There is also now evidence that dexamethasone can cause abortion in the dog. Glucocorticoids generally have teratogenic effects during early pregnancy and should be avoided in the breeding animal if possible.

Severe Sequelae

Glucocorticoid overdosage may lead to severe and potentially fatal toxicity associated with sepsis resulting from GI tract disruption and immune suppression in the dog (Bellah et al., 1989). Although pituitary pars intermedia dysfunction and hypercortisolemia have been associated with an increased incidence of laminitis in horses, a review has suggested that glucocorticoid-induced laminitis is relatively rare (Cornelisse and Robinson, 2013). Glucocorticoids appear to potentiate the action of catecholamines in the equine digit. Because this constriction is more pronounced on venous beds than on arteriolar beds, the net result is often digital congestion and edema (Barragry, 1994). The enzyme 11-β steroid dehydrogenase type 1 (11-β HSD1) is occasionally elevated in hoof lamellar tissues during conditions of glucocorticoid excess, resulting in a greater tendency toward metabolically active glucocorticoids. Glucocorticoids have been postulated to reduce digit perfusion by a direct action on smooth muscle as well as by inducing insulin resistance. Endothelial damage associated with elevated glucose leads to increased production of endothelin-1 and a reduction in nitric oxide (NO) production tending to promote vasoconstriction (Johnson et al., 2004).

Principles of Rational Glucocorticoid Therapy

Because of their wide ranging and nonspecific effects, reports of the clinical use of glucocorticoids are replete with pragmatic recommendations regarding dosage, duration of therapy, and severity of side effects. Much has been adopted from clinical use in humans, and much more is known about the fine points of glucocorticoid therapy in dogs and cats than in other species. In the following discussion, most of the specific comments will apply to the use of glucocorticoids in the dog; however, when available, appropriate information for other species will be mentioned. It is important to recognize that glucocorticoids rarely cure disease. With the possible exception of spontaneous glucocorticoid deficiency, they are used to try to suppress clinical signs long enough for a condition to run its natural course (Fauci, 1976; Ferguson, 1985a, 1985b; Melby, 1974; Wilcke and Davis, 1982).

A well-known endocrinologist named Thorn proposed, in 1966, that physicians ask themselves the following questions *before* using glucocorticoid therapy (Hench, 1952). A similar approach is proposed for use in animals:

1) How serious is the underlying disorder?
2) How long will therapy be required?
3) Is the patient predisposed to any complications of glucocorticoid therapy?
4) What is the anticipated glucocorticoid dosage?
5) Which glucocorticoid preparation should be used?
6) Have other types of treatment been used to minimize glucocorticoid dosage and side effects?
7) Is an alternate-day regimen indicated?

These questions may help develop guidelines for practical and rational glucocorticoid therapy.

How Serious Is the Underlying Disorder? How Long Will Therapy Be Required?

The following general principles should be considered when glucocorticoid therapy is employed:

Diagnose the disease first, if possible. Glucocorticoids are generally only palliative and do not provide a true cure for any disease. In addition, if used before all reasonable diagnostic tests have been completed, they may mask signs of underlying disease and complicate specific diagnosis and therapy. Though a definitive diagnosis is not always possible, a presumptive diagnosis should be proposed (Ferguson, 1985a, 1985b).

Classify the disorder into one of the following categories of glucocorticoid therapy, according to a definitive or presumptive diagnosis: physiological replacement, intensive short-term, antiinflammatory and antiallergic, immunosuppressive, and chronic palliative. Each of these usage classifications will be discussed in more detail. By using these classifications, the clinician clearly defines the goal of therapy and can choose a starting dose and formulation appropriate for the disorder.

Use glucocorticoids to accomplish specific objectives. It is important to decide on the therapeutic endpoint *before* therapy is started in order to objectively assess efficacy and determine the smallest effective dose. For example, in treatment of a dog with autoimmune hemolytic anemia, the goal for initial glucocorticoid therapy might be to raise the hematocrit from 10% to 25%. In a horse with chronic obstructive pulmonary disease (“heaves”), the goal might be to suppress the allergic reaction for 1–2 weeks in order to allow the owners to change the feeding regimen to eliminate the offending allergen. By defining a therapeutic objective, the clinician can then judge the efficacy of a treatment protocol and decide when the glucocorticoid dose should be altered or alternative therapy chosen.

The length of therapy should also be anticipated. For example, immunosuppressive therapy generally requires several months of glucocorticoid use. Accordingly, a plan for instituting and later decreasing the dose should be considered from the outset. In such a case, intermittent or alternate-day therapy would not be appropriate, and an intermediate- or long-acting glucocorticoid could be used.

Is the Patient Predisposed to Any Complications of Glucocorticoid Therapy?

Because many of the therapeutic effects of glucocorticoids are nonspecific, clinicians should anticipate the impact on the patient of the previously outlined complications of glucocorticoid use. In doing so, the risk/benefit ratio of using these potent agents is considered.

What Is the Anticipated Glucocorticoid Dosage? Which Glucocorticoid Preparation Should Be Used?

It is important to understand the relative potency and, perhaps more important, the relative *duration of action* of a glucocorticoid preparation, because the duration of antiinflammatory effects usually parallels the duration of effects on the HPAA. Success with alternate-day therapy, more commonly applied in small-animal practice, depends on selecting a glucocorticoid preparation with slightly longer antiinflammatory or immunosuppressive (beneficial) actions than HPAA-suppressive effects. *Dosages of glucocorticoids are derived by trial and error and should be constantly reevaluated.* Due to the aforementioned hazards of long-term daily glucocorticoid use, intermittent or alternate-day therapy is preferred when long-term use is necessary. As shown in Table 29.3, short- or intermediate-acting formulations, generally given orally, are most appropriate and safe for long-term use and alternate-day therapy. The goal of rational therapy is to maintain a condition in remission at the lowest effective glucocorticoid dosage (Fauci, 1976; Feldman and Nelson, 2004; Ferguson, 1985a, 1985b; Wilcke and Davis, 1982).

Classes of Glucocorticoid Usage

Physiological Replacement Therapy

Replacement therapy involves use of glucocorticoids in amounts similar to those of the naturally occurring glucocorticoids (cortisol in virtually all domestic species) from the adrenal gland. Ideal replacement therapy should mimic the adrenal gland’s hormonal output under basal conditions, with doses increasing if the animal is stressed by illness or surgery. Practically, this ideal is never achieved; however, the following regimens have been used successfully in adrenalectomized and addisonian dogs and cats. As a general rule, animals produce approximately 1 mg/kg of cortisol (hydrocortisone) every day.

It is not rational to employ alternate-day or intermittent glucocorticoid replacement therapy in a glucocorticoid-deficient animal, because the animal's metabolic well being depends upon the presence of glucocorticoids *every day.* Therefore, physiological replacement therapy is aimed at providing a small daily amount of glucocorticoid. Physiological replacement therapy is rarely indicated or applied in large animals. In small animals, hydrocortisone or cortisone at 0.2–1 mg/kg/day or, more commonly, equipotent amounts of prednisolone or prednisone at 0.1–0.2 mg/kg/day once daily orally are indicated. Unlike in man, dogs and cats do not appear to have diurnal variation in cortisol concentrations (Kemppainen, 1986). Therefore, the timing of the single daily dosage would not appear to be critical, other than that it be provided approximately the same time each day. Because stress results in higher adrenal output of glucocorticoids, this pattern should be mimicked; in general, in moderate stress, give 2–5 times the physiological dosage, and in severe stress (e.g., surgery), administer 5–20 times this dosage until the stressful experience has ended (Ferguson, 1985a, 1985b).

Intensive Short-Term and Shock Therapy

The effects of glucocorticoids in all forms of shock are still controversial; however, some evidence suggests that early treatment (probably about 4 hours postinduction in dogs) may lead to increased survival, particularly in hemorrhagic and septic shock. The nature of the formulation (particularly the ester) may affect the speed of cellular entry of glucocorticoids during shock; however, other conclusions have also been reached (Ferguson, 1985a, 1985b; Ferguson et al., 1978; Sprung et al., 1984; Wilcke and Davis, 1982; Wilson, 1979).

Glucocorticoids improve hemodynamics and enhance survival in canine models of endotoxic and hemorrhagic shock. However, therapy for shock should also include aggressive fluid therapy. Septic (endotoxic) shock is the most responsive form to glucocorticoid therapy; however, although human trials have shown improved short-term survival, most patients succumbed to chronic septicemia later (Sprung et al., 1984). Suspected endotoxic shock should be treated with fluid therapy and a broad-spectrum antimicrobial, with or without glucocorticoids. Glucocorticoids and antibiotics were synergistic when given within 2 hours of induction of septic shock in baboons.

The potential detrimental effects of massive doses of glucocorticoids should always be considered. However, proponents of glucocorticoid therapy for shock point out that short-term (~48 hour) glucocorticoid therapy has few negative effects, and the positive effects far outweigh the risks. Most human patients with sepsis survive beyond the acute stages of endotoxemia but succumb later to chronic septicemia (Sprung et al., 1984). Certainly, the immunosuppressive effects of glucocorticoids make their use contraindicated during chronic sepsis, and those supporting glucocorticoid use in septic shock do not generally advocate use other than during the early acute hypotensive state. Opponents to glucocorticoid use generally are not convinced that experimental studies in anesthetized animals adequately duplicate clinical situations and do not believe even short-term treatment to be innocuous, because of immunosuppressive effects (Wilcke and Davis, 1982).

Antiinflammatory and Antiallergic Therapy

A large proportion of glucocorticoid use in veterinary practice is designed to combat inflammation or allergy. Unfortunately, many such diseases are difficult to definitively diagnose. Therefore, misuse of glucocorticoids is not uncommon in this category. Examples of antiinflammatory and antiallergic use of glucocorticoids include symptomatic treatment of pruritic dermatoses, allergic pulmonary disease, and allergic gastroenteritis. Guidelines for antiinflammatory and antiallergic dosages vary from species to species. Prednisolone or prednisone is most commonly used in small animals at 0.55 mg/kg q 12 h given orally for induction, and then at 0.55–2.2 mg/kg every other day for maintenance. Although all dosages should be adjusted according to effect, a general, but undocumented, observation has been that cats require approximately twice the glucocorticoid dosage that dogs require to manage a similar condition. Methylprednisolone acetate may also be administered subcutaneously or intramuscularly at 1.1 mg/kg every 1–3 weeks; however, use of depot products brings the distinct disadvantage that the drug dosage cannot be stopped or reduced. Other long-acting injectable products and their duration include prednisolone acetate, 1–2 days; dexamethasone in propylene glycol, 1–7 days; triamcinolone acetonide, 3–7 days; and betamethasone valerate, 7–60 days. For practical reasons of cost and potency, dexamethasone is the most commonly used glucocorticoid in large animals.

Immunosuppressive Therapy

Protracted glucocorticoid use generally is required for immunosuppression. Therefore, use of a glucocorticoid with well-documented side effects and efficacy is recommended. It is important to use the highest recommended dosage until clinical signs abate. After that point, the dosage may be decreased in increments. In general, in small animals, the dosage may be decreased until the equivalent prednisolone dosage of 1.1 mg/kg is being given on alternate days (Aucoin, 1982; Ferguson, 1985a, 1985b). Long-term side effects of alternate-day therapy are few, and the dosage rarely must be decreased further. Therapy should not be discontinued until the autoimmune disease is in remission for 2–3 months; otherwise, signs are likely to recur (Aucoin, 1982; Fauci, 1978; Ferguson, 1985a, 1985b).

Glucocorticoids provide very nonspecific protective benefits: "The hormone appears not to extinguish the fire or to act like a carpenter to repair the damage of the fire. Instead, it appears to 'dampen the fire,' or to provide, as it were, an asbestos suit behind which the patient, like some Biblical Shadrach, Meshach or Abednego, protects his tissues from the fire. If this protection is removed prematurely, before the fire has spent itself, the patient and his tissues will react again to the burning. But, if the protection is not discarded until the natural duration of the fire is over, the patient remains largely free of symptoms and apparently 'well' " (Hench, 1952). Unlike other immunosuppressants, glucocorticoids do not significantly inhibit antibody production by B lymphocytes until possibly very high dosages. If glucocorticoids provide incomplete remission of an immune-mediated disorder, other immunosuppressant agents such as the alkylating agent cyclophosphamide may be added to complement the effects of glucocorticoids. Furthermore, if side effects of the glucocorticoids are too great, other immunosuppressants may be added to the regimen. If no clinical response is obtained with glucocorticoid therapy alone, addition of other immunosuppressants is less likely to succeed. Immune-mediated thrombocytopenia and autoimmmune hemolytic anemia are examples of diseases treated with immunosuppressive doses of glucocorticoids.

In small animals, immunosuppression is generally accomplished with prednisolone at 2.2–6.6 mg/kg or the equipotent dosage of dexamethasone at 0.33–1.1 mg/kg q 12 h for induction, and prednisolone at 1.0–2.2 mg/kg every other day for maintenance. Note that, because its duration of action exceeds 24 hours, dexamethasone is acceptable for induction but not for alternate-day maintenance therapy (Ferguson, 1985a, 1985b).

The adverse effects of chronic immunosuppressive doses of glucocorticoid use can be serious. Therefore, clinicians should eventually attempt to maintain a satisfactory therapeutic result with the smallest possible dose of glucocorticoids on alternate days if possible. Nonsteroidal immunosuppressants drugs (e.g., cyclosporine or mycophenolate for immunosuppression) may be used as alternative or adjunctive therapy as necessary.

Chronic Palliative Therapy

Historically, before a wider array of nonsteroidal antiinflammatories became available in the early 1990s, glucocorticoids were commonly used when nonsteroidal antiinflammatory therapy no longer succeeded at managing chronic conditions, such as arthritis in most species or hip dysplasia in dogs. If nonsteroidal analgesics are not satisfactory, glucocorticoids may be used on an intermittent or alternate-day basis. It is important not to administer glucocorticoids erratically, as rapid withdrawal may precipitate signs of lameness or stiffness ("pseudorheumatism") (Fauci, 1976, 1978; Ferguson, 1985a, 1985b).

Alternate-Day Therapy

Side effects of long-term glucocorticoid use can be dramatically reduced by alternate-day therapy. Allowing the HPAA to recover on "off" days provides greater safety if therapy should suddenly be discontinued. Successful use of alternate-day therapy depends upon the therapeutic effects lasting longer than HPAA-suppressive effects. As a result, this approach is not successful for all diseases. Also, true alternate-day therapy is rarely applied to large-animal cases because it is less practical to give large animals agents with low or intermediate potency like prednisolone. Weaning of more-potent agents such as dexamethasone often is accomplished by extending the between-dose interval to as long as 3–4 days in large animals; however, this is technically not considered alternate-day therapy, because full recovery of the HPAA is not allowed on alternate days (Barragry, 1994; Ferguson, 1985a, 1985b).

Several common pitfalls of alternate-day therapy should be avoided. Alternate-day therapy is rarely, if ever, effective as primary therapy. It is usually first necessary to use daily therapy to achieve the desired clinical effect. Alternate-day therapy with long-acting glucocorticoids is not rational. The change to alternate-day glucocorticoid use ideally should be gradual, particularly after prolonged high-dosage therapy. Rapid change to alternate-day use may result in signs of glucocorticoid withdrawal. Finally, alternate-day glucocorticoid therapy may fail if used exclusively; supplemental use of alternative therapy should be considered, particularly on "off" days (Fauci, 1978; Ferguson, 1985a, 1985b).

In inflammatory joint disease caused by infectious or immune-mediated conditions, early aggressive therapy is usually necessary to limit subsequent joint dysfunction. Of course, the primary therapy for immune-mediated arthritis, immunosuppression, can jeopardize the health of patients with infectious arthropathies. So before initiating immunosuppressive therapy, follow a thorough diagnostic plan to exclude infectious causes (Michels and Carr, 1997).

Changing to Alternate-Day Therapy

Because glucocorticoid administration to dogs for longer than 2 weeks generally results in significant loss of adrenal functional reserve, for the sake of this discussion, administration of greater than 0.5 mg/kg/day of prednisolone or an equipotent dosage of a more potent drug for longer than 2 weeks should be considered chronic therapy (Chastain and Graham, 1979). However, when 0.5 mg/kg q 12 h (an antiinflammatory dosage) was administered to dogs for 35 days and stopped abruptly, it took less than 2 weeks for the HPAA to totally recover (Moore and Hoenig, 1992). This contrasts drastically with the experience in humans, where normalization of cortisol secretion and pituitary function may take as long as 6–9 months (Fauci, 1976, 1978). Similar studies have not been performed in large-animal species.

There is no "correct" way to taper an animal from glucocorticoids. The following guidelines are suggested. If the glucocorticoid dosage is large (>1 mg/kg/day prednisolone or an equivalent) or therapy prolonged (>2 weeks in duration), some process of gradually reducing the steroid dosage (i.e., weaning) is indicated. One highly conservative approach is to double the glucocorticoid dose for "on" days and taper the dose for "off" days by 25% per cycle (a cycle may vary from 1 day to several weeks). Another conservative method includes increasing the dose for "on" days by the same amount as the dose for "off" days is decreased. Practical experience indicates that, in many canine patients, rapid tapering has few recognizable side effects unless the animal is severely stressed. Subtle adverse effects may be missed unless the owners and clinician are vigilant. Patient tolerance defines the success of any change. If therapy is for less than 2 weeks, it is probably safe to rapidly taper the dog and have no therapy on "off" days. If clinical signs are observed on "off" days, supplement with a replacement dose of glucocorticoids on those days, or add nonsteroidal therapy. If alternate-day therapy is ineffective, use of a single dose each morning (to mimic diurnal variation) may also minimize adverse effects.

Withdrawal from Glucocorticoids

The identification of clinical signs of glucocorticoid deficiency may be very difficult. Animals cannot complain of minor aches and pains or of mood swings as do people being withdrawn from glucocorticoids. Signs of glucocorticoid withdrawal may include dullness, depression, decreased exercise tolerance, incoordination, unthriftiness and weight loss, loose stools, and behavioral changes. Significant adrenocortical suppression occurs in dogs within 2 weeks of initiating daily glucocorticoid therapy. Therefore, it is reasonable to assume that dogs and cats may require supplementation of glucocorticoids during episodes of stress, such as illness or surgery, particularly if signs of glucocorticoid withdrawal are present. It should be emphasized that short-term use of glucocorticoids in physiological amounts has few risks despite the evidence that these "physiological" quantities significantly suppress the HPAA, resulting in adrenal atrophy (Byyny, 1976; Chastain and Graham, 1979).

Test of Adrenal Reserve

Laboratory tests usually are not necessary to diagnose most cases of iatrogenic adrenal insufficiency; a good history usually indicates the cause of the problem. Occasionally, in the absence of an accurate history or when surgery is considered for a dog with suspected adrenal insufficiency, an ACTH stimulation test is performed to test adrenal functional reserve.

ACTH stimulation test protocol: A venous blood sample is collected in heparin tubes and centrifuged within 15 minutes, with the plasma immediately frozen for later plasma cortisol determination. A dose of 0.25 mg (25 units or an entire vial, though less will work, regardless of the animal's size) of synthetic ACTH (Cortrosyn, Organon) is given intravenously or intramuscularly. For the dog or horse, a postinjection venous blood sample, handled as for the preinjection sample, is collected 1 hour after IV Cortrosyn injection. If cost is a concern, valuable information on adrenal secretory reserve can be obtained by giving ACTH and collecting a blood sample only at the appropriate time after injection. Blood samples are then assayed by a clinical pathology laboratory for plasma cortisol levels. In cats, peak cortisol concentrations should be measured 30 minutes after intravenous ACTH administration.

Interpretation of test results: Healthy unstressed animals have basal plasma cortisol levels in the normal range that increase by 50–100% after ACTH stimulation. Some dogs, after chronic glucocorticoid treatment, may have normal basal cortisol levels but a post-ACTH increase of less than 50%. Such dogs do well until stressed and then require glucocorticoid supplementation (see Section Glucocorticoid Supplementation During Stress). Other dogs may have low basal levels as well as low post-ACTH cortisol levels, indicating a need for continued regular glucocorticoid supplementation as well as additional glucocorticoids during periods of stress (Ferguson, 1985a, 1985b).

Glucocorticoid Supplementation During Stress

Animals with marginally adequate or deficient adrenal function require supplementation of glucocorticoids during periods of stress. In situations of minor stress, such as minor surgery, general anesthesia, a minor illness, or even a visit to the veterinarian, glucocorticoid can be given to avoid collapse and other complications. For example, hydrocortisone or cortisone can be given at 2–5 mg/kg or prednisolone or prednisone at 0.4–1.0 mg/kg. In severely stressful situations, such as in severe illness or major surgery, higher dosages may be necessary. In preparing an animal for major surgery (including adrenalectomy), prednisolone acetate can be given intramuscularly at 0.4–2 mg/kg the night before and the morning of surgery. Alternatively, or in addition, 100–300 mg hydrocortisone can be given by IV drip. These large doses should be gradually reduced within

3–5 days to maintenance levels unless there are complications (Ferguson, 1985a, 1985b).

Considerations for Combination or Sequential Glucocorticoids and Nonsteroidal Antiinflammatory Drugs

Some clinicians have advocated for a "washout time" between switching between two NSAIDs or between a glucocorticoids and a NSAID. While there is good evidence that concurrent administration of a NSAID and a corticosteroid exacerbates GI lesions (Dow et al., 1990; Boston et al., 2003), there is no solid evidence documenting the logic for drug-free intervals moving from one NSAID to another or between NSAIDs and glucocorticoids. Nonetheless, some rational guidelines should be considered. Undoubtedly, if a COX-2-inhibiting drug or glucocorticoid administration follows a known gastroduodenal injury caused by an NSAID or glucocorticoid, there may be increased risk of delayed healing and further injury. However, administration of aspirin and other COX-nonselective drugs may pose a particular risk because they trigger the COX2-dependent synthesis of 15(R)-epi-lipoxin A4 also known as aspirin-triggered lipoxin (ATL), a substance that acts to ameliorate injury to the stomach caused by reduction in protective prostaglandin E1. Simultaneous or subsequent inhibition of COX-2 activity by a selective COX-2 inhibitor or a conventional nonsteroidal antiinflammatory drug (NSAID) removes the formation of ATL by aspirin. In the absence of the protective effects of ATL, the extent of gastric damage is increased. Therefore, coadministration of aspirin and a selective cyclooxygenase 2 inhibitor results in more gastric damage than that induced following administration of either drug alone (Wallace and Fiorucci, 2003). There is indirect evidence that ATL develops in the dog; older studies have shown that after 1–2 weeks of aspirin, GI lesions resolved despite continued administration. High doses of aspirin may over-ride the process of adaptation (Sennello and Leib, 2006). Finally, be sure to consider endocrine effects of glucocorticoids and wean, do not remove precipitously. When serious pain management is needed between NSAIDs, consider other pain-relieving agents: for example gabapentin, fentanyl, codeine, or tramadol.

Miscellaneous or Special Usages

Topical and intralesional usage: Topical and intralesional glucocorticoid administration is occasionally used to manage localized lesions of the skin (see Chapter 47). Despite the route of administration, systemic effects, including suppression of the HPAA, should be expected. Acute inflammatory conditions such as pyotraumatic dermatitis and urticaria are usually managed with nonocclusive, nonheating glucocorticoid preparations. However, chronic conditions are most commonly managed with penetrating glucocorticoid creams and ointments. The potent fluorinated bases such as betamethasone, dexamethasone, triamcinolone, and fluocinolone are the most commonly preferred. The topical, intralesional, or intraarticular use of compounds (e.g., prednisone, cortisone) requiring hepatic activation is of questionable value (Coppoc, 1984; Glaze et al., 1988; Kemppainen, 1986; McDonald and Langston, 1994; Scott, 1982; Scott and Greene, 1974; Wilcke and Davis, 1982).

Intraarticular administration: Intraarticular glucocorticoids have been utilized to manage the orthopedic conditions of traumatic arthritis, myositis, bursitis, and tendinitis. Used primarily in equine medicine to manage joint inflammation and pain, the practice of intraarticular glucocorticoid therapy is controversial and potentially dangerous. Glucocorticoids tend to reduce the pain for a working animal but also diminish chondrocyte collagen and synovial fluid production. The benefits cited for this practice include the reduction of proteolytic enzymes in joint fluid and reduction of joint swelling and discomfort. The hazards include encouragement of further mechanical damage, loss of joint proteoglycan, development of septic arthritis, and inhibition of chondrocyte and osteoblast activity, with the end result being joint or bone breakdown. Intraarticular administration of glucocorticoids leads to systemic absorption and HPAA suppression (Barragry, 1994).

Special considerations for intraarticular glucocorticoid usage in horses: The rationale for intraarticular administration in the horse includes the following: (i) the size of the animal, which requires a large systemic dose of drug to be administered, (ii) the higher incidence of acute arthritis conditions in these animals, and (iii) the adverse systemic effects of corticosteroids. This route is effective for the treatment of synovitis and capsulitis; however, imaging techniques should confirm that there is no prior structural damage, and strict aseptic technique is mandatory. Chronic degenerative joint conditions tend to respond less well than acute ones. Surgery following glucocorticoid therapy can be problematic as corticosteroids slow the rate of healing as described above. Additional forced exercise may compound the joint damage; therefore, at least 3 months should be given to allow damaged cartilage and subchondral bone to heal (Gabel, 1977). In some cases, complete breakdown of the affected joint has occurred during the race following intraarticular glucocorticoid treatment (Tobin, 1979; Upson, 1978). It has been suggested that the rational usage of corticosteroids are effective in prolonging the performance career of carefully selected equine patients, particularly those in which surgical intervention and/or rest will not be of benefit (McKay and Milne, 1976). Furthermore, as previously discussed, glucocorticoid administration is a minor a risk factor for laminitis. In summary, the intraarticular route of administration must be used judiciously (Barragry, 1994). Biochemical and biomechanical properties of osteochondral allograft tissue from juvenile cattle and dogs may be enhanced by the addition of dexamethasone to culture media. These findings may translate to longer shelf life of preserved osteochondral allograft transplantation tissue and increased clinical availability of grafts (Bian et al., 2010). In equine cartilage explants, glucocorticoids had no significant effect on expression of genes studied. Although methylprednisolone acetate and triamcinolone have been shown to have differential effects on chondrocyte metabolism, it is believed that these differences are due to posttranslational and not genomic mechanisms (Caron et al., 2013).

Ophthalmic applications: Glucocorticoids are used topically and subconjunctivally to manage inflammatory conditions of the eye, including retinitis, choroiditis, optic neuritis, and orbital cellulitis (see Chapter 49). These agents stabilize the blood–aqueous and blood–retinal barriers, reducing the leakage of protein into the aqueous that accompanies edema and inflammation. Topical ophthalmic administration of glucocorticoid preparations is also employed to minimize neovascularity and, by inhibiting fibroblast activity, to inhibit corneal scarring, pigmentation, and the formation of synechia.

Topical glucocorticoids are available in solutions, ointments, and suspensions. Penetration, therefore, is determined by two factors: the chemical composition of the base and the vehicle. Except for prednisolone acetate, the alcoholic forms of cortisone, hydrocortisone, and prednisone penetrate the cornea more readily than the acetate ester forms.

Subconjunctival administration of glucocorticoids is used to manage conjunctivitis, keratitis, scleritis, and anterior uveitis. However, it is not the route of choice for diseases of the posterior segment. High concentrations of glucocorticoids can be achieved with subconjunctival administration because the sclera is very permeable to steroids.

Glucocorticoids are contraindicated in corneal ulcers because they slow the process of reepithelialization of the cornea. Furthermore, glucocorticoids enhance the activity of collagenase, which is produced by bacteria like *Pseudomonas* and by leukocytes, and may contribute to the development of "melting" corneal ulcers (Brightman, 1982; Glaze et al., 1988; McDonald and Langston, 1994). Iatrogenic Cushing's syndrome has resulted from topical ophthalmic preparations (Murphy et al., 1990).

Neurological applications: Neurological applications of glucocorticoid therapy are numerous. Potent antiinflammatory action is often necessary to manage acute spinal or CNS trauma, acute cervical or lumbar pain, vestibular disease, acute traumatic and some chronic peripheral neuropathies, polymyositis, and CNS neoplasia (Ferguson, 1985a, 1985b; McDonald and Langston, 1994; Metz et al., 1982; Wilcke and Davis, 1982; Meintjes et al., 1996).

Newer Preparations

Selective glucocorticoid receptor agonists: Given the previously discussed existence of cGR isoforms, future drug development is likely to include qualitatively new drugs, such as selective glucocorticoid receptor agonists (SEGRAMs), but none have become clinically available yet. Several such compounds are under development and are showing promising results. The advantage would be to allow selective therapeutic antiinflammatory benefits associated with transrepression of AP-1 and NF-κB stimulated synthesis of inflammatory mediators, while adverse effects were associated with the transactivation of genes involved in metabolic processes (Buttgereit, 2004; Stahn et al., 2007).

21-Aminosteroids (lazaroids): Neuroprotective effects of GCs at high dosages are independent of GR interaction. 21-aminosteroids have been developed as highly lipophilic drugs, which retain neuroprotective (antilipid peroxidase) effects, in part by reducing fluidity of membranes. They may also appear to scavenge hydroxyl radicals and reduce eicosanoid mediators and tumor necrosis factor, without metabolic effects such as alterations in hematology, glucose, ACTH, or cortisol. Although still largely in development as human pharmaceuticals, their projected use is in acute nervous system trauma, membrane protectants in most forms of shock, and in chronic neurodegenerative diseases (Buttgereit et al., 2004).

Nitrosteroids: This class of glucocorticoids releases low levels of nitric oxide (NO) and it is believed that posttranslational modification of GR by tyrosine nitration is responsible for its enhanced antiinflammatory activity and reduced side effects. Further studies are required in the clinical setting and none have been performed to date in domestic animals (Buttgereit et al., 2004).

"Soft" steroids: Budesonide, ciclesonide, and loteprednol etabonate are examples of "soft" steroids that are designed for delivery near to their site of action to exert their effect, and then undergo metabolism to inactive metabolites. This retrometabolic drug design has the goal of reducing systemic side effects. Budesonide has been used as an orally administered product in dogs and cats primarily for the treatment of inflammatory bowel disease (Pietra et al., 2013) and for feline asthma (Chang et al., 2011). It is absorbed in the intestine and undergoes first-pass metabolism to an inactive metabolite. Approximately 15 times more potent than prednisolone, it still has shown systemic glucocorticoid side effects. It also has been evaluated for topical dermatological therapy (Ahlstrom et al., 2013; Olivry and Bizikova, 2013). Ciclesonide is a new-generation inhaled corticosteroid, which is hydrolyzed by esterases in the upper and lower airways to its pharmacologically active metabolite desisobutyrl-ciclesonide. In human patients, it is used for treating asthma and allergic rhinitis. Limited studies in dogs have shown long-lasting antiallergic (antiasthmatic) effects without influencing the hypothalamic–pituitary axis. Loteprednol is an inactive-metabolite soft steroid that has been used in human medicine as an ophthalmic or inhaled product (Belvisi and Hele, 2003; Bodor and Buchwald, 2006).

Mineralocorticoids

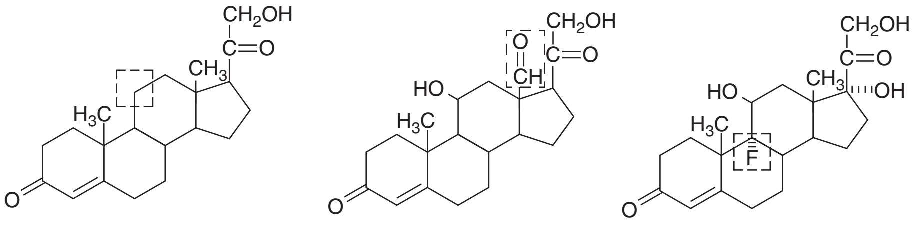
History

In 1855, Thomas Addison first described the clinical manifestations of primary adrenal insufficiency (Addison, 1855); however, it was not until 1929 that crude extracts of adrenal cortex were used in clinical treatment trials of patients with Addison's disease (glucocorticoid and mineralocorticoid deficiency) (Rogoff and Stewart, 1969). In 1937, the adrenocortical steroid 11-desoxycorticosterone was finally produced and made available for treatment of Addison's disease (Thorn et al., 1942). It was able to prevent the urinary sodium loss and was the major therapy for the treatment of Addison's for several years. It was not until the early 1950s that aldosterone was discovered, and it was established that this hormone was involved in water and electrolyte balance (Luetscher, 1956; Simpson et al., 1954). Aldosterone was isolated and synthesized in the mid-1950s (Ham et al., 1955; Simpson et al., 1954). It is by far the most potent of the naturally occurring corticosteroids with regard to water and electrolyte balance.

Secretion and Mechanism of Action

Almost all naturally occurring and synthetically derived corticoids have both mineralocorticoid and glucocorticoid activity but are usually designated on the basis of their predominant activity. Mineralocorticoid hormones are secreted by both the zona glomerulosa and zona fasciculata. The zona glomerulosa produces aldosterone and 18-hydroxycorticosterone under the major control of angiotensin II, while the zona fasciculata produces mainly desoxycorticosterone, 18-hydroxydeoxycorticosterone, and corticosterone under ACTH regulation (Mantero et al., 1990). Most of our knowledge of mineralocorticoid action is derived from studies on the classical target organ for mineralocorticoids, the kidney. Mineralocorticoids bind to a specific receptor, the mineralocorticoid receptor (MR). The MR is a member of the steroid/ thyroid/ retinoid/ xenobiotic/ orphansuperfamily of intracellular receptors, which are ligand-dependent transcription factors. In the absence of hormone the MR resides predominantly in the cytoplasm (Fejes-Tóth et al., 1998) complexed with heat-shock proteins (HSPs) (Couette et al., 1998). Upon hormone binding, there is a conformational change in the receptor's structure, the HSPs dissociate, and the receptor migrates to the nucleus where it binds to the promoter region of target genes and regulates transcription from these genes (Rogerson and Fuller, 2000). The human MR was cloned in 1987 by Arriza (Arriza et al., 1987), and its mRNA was demonstrated in various tissues such as kidney, hippocampus, pituitary, heart, and spleen. The naturally occurring MR ligands are predominantly aldosterone and deoxycorticosterone, although other steroids such as progesterone show high-affinity binding to MR. Aldosterone is the most potent regulator of electrolyte excretion and is essential for life (Sutanto and deKloet, 1991). It is believed to exist in at least five isoforms, which may have different biological activity. Deoxycorticosterone is also a naturally occurring mineralocorticoid with similar binding patterns to MR as aldosterone.

Mineralocorticoids exert their effect in target tissues through interaction with the MR receptor. Hormone-receptor complex binds to chromatin and induces transcription of mRNA, which is subsequently translated to generate proteins. All physiological actions of mineralocorticoids depend on gene activation and new protein synthesis (Johnson, 1992). A two-step model for mineralocorticoid action has been proposed. Changes in membrane electrolyte transport in the kidney are fast (within minutes) and involve the stimulation of Na^+,K^+-ATPase and activation of the Na^+/H^+ exchanger, which leads to Na^+ influx into the cell at the expense of H^+ and K^+, while de novo synthesis of Na^+,K^+-ATPase is a late response (within hours or days). Aldosterone also affects the epithelial Na channel and aldosterone rapidly induces the transcription of gene(s) encoding regulatory protein(s) that increase epithelial Na channel activity in the renal collecting duct and distal colon (Snyder, 2002; Pearce and Kleyman, 2007). The net effect of mineralocorticoid action is, therefore, Na^+ retention, proton excretion, and K^+ excretion (Wehling et al., 1991; Wehling et al., 1992). The addisonian patient typically has hyponatremia, hyperkalemia, and metabolic acidosis.



Deoxycorticosterone Aldosterone Fludrocortisone

Figure 29.9 Structures of common mineralocorticoid drugs used in veterinary medicine. The key elements of structure differing from cortisol are identified by the outlined areas.

Preparations and Properties

The following preparations are currently available for the treatment of mineralocorticoid deficiency:

1) Desoxycorticosterone pivalate (DOCP), a long-acting ester of desoxycorticosterone acetate (DOCA), is available as a sterile suspension for intramuscular injection (Percorten-V$^{\text{TM}}$, Novartis). It has been approved for use in the dog.
2) Fludrocortisone acetate is available for oral use in generic form only. It is manufactured as 0.1-mg tablets (Global Pharmaceuticals), and also from compounding pharmacies in custom-made doses. It has a half-life of approximately 8 hours in humans. Fludrocortisone also has substantial glucocorticoid activity.

Note: Aldosterone is only available for research, not for therapeutic use. The structures of the mineralocorticoid compounds are shown in Figure 29.9.

Therapeutic Use

Historically, DOCA was the treatment of choice for the mineralocorticoid deficiency in acute primary adrenal failure but the drug has been discontinued for many years. For the acute crisis situation or maintenance therapy, either DOCP or fludrocortisone acetate may be used. An initial starting dose of DOCP of 2.2 mg/kg of body weight every 25 days has been recommended (Lynn et al., 1993) for dogs. In the cat a dose of 12.5 mg every 3–4 weeks has been recommended (Greco and Peterson, 1989). These doses and the time intervals of injections may be adjusted depending on the response to therapy as measured by serum Na$^+$ and K$^+$ concentrations.

Fludrocortisone acetate must be administered daily for the treatment of hypoadrenocorticism. In the dog the dose is 0.1–0.5 mg orally twice daily or 0.01 mg/kg divided every 12 hours orally. In the cat the dose is 0.1–0.2 mg divided every 12 hours orally. The dose may have to be adjusted based on weekly electrolyte measurements. Once the animal is stable, rechecks, including serum Na$^+$ and K$^+$ measurement, should be made on a monthly basis. Dogs metabolize this drug rapidly and high doses may be necessary even for less-than-optimal results (Hoenig and Ferguson, 1991b).

Side Effects

Adverse effects of mineralocorticoid replacement therapy are rare but may include hypokalemia, hypernatremia, muscle weakness, and hypertension, particularly in patients with borderline renal disease (Hoenig and Ferguson, 1991b). Because fludrocortisone also has glucocorticoid activity, animals on large doses may show signs of glucocorticoid excess (Lynn et al., 1993). It is important that any fluid deficits be corrected prior to treatment with mineralocorticoids.

Adrenolytic Drugs and Steroid Synthesis Inhibitors

Therapy for Hyperadrenocorticism

Spontaneous hyperadrenocorticism is characterized by excess secretion of the glucocorticoid cortisol. In 85–90% of cases in the dog and the majority of cases in the horse, the primary species suffering from this condition, the cause is excess ACTH production by the pituitary. The term “Cushing’s disease” is the term used when the adrenal glands are bilaterally hypertrophied and producing excess cortisol in response to overproduction of ACTH by the pituitary corticotrophs. In the horse, an intermediate lobe pituitary tumor is the most common cause. Accordingly, in dog and horse, there have been attempts to reduce ACTH production with dopaminergic compounds (like bromocryptine mesylate or pergolide mesylate) or the antiserotoninergic agent cyproheptadine. The experience with these agents has largely been unsatisfactory in the dog due to toxicity and lack of efficacy. In the horse, pergolide seems to be a more effective treatment than cyproheptadine or bromocryptine (Schott, 2002).

Low hypothalamic dopamine concentrations have been observed in dogs with pituitary-dependent hyperadrenocorticism. As such, dopamine deficiency has been proposed as an underlying etiology for this condition. L-Deprenyl (Anypril®, Pfizer Animal Health), a monoamine oxidase B enzyme inhibitor that inhibits the breakdown of dopamine, has been approved for the treatment of uncomplicated pituitary-dependent hyperadrenocorticism in the dog (Figure 29.10).

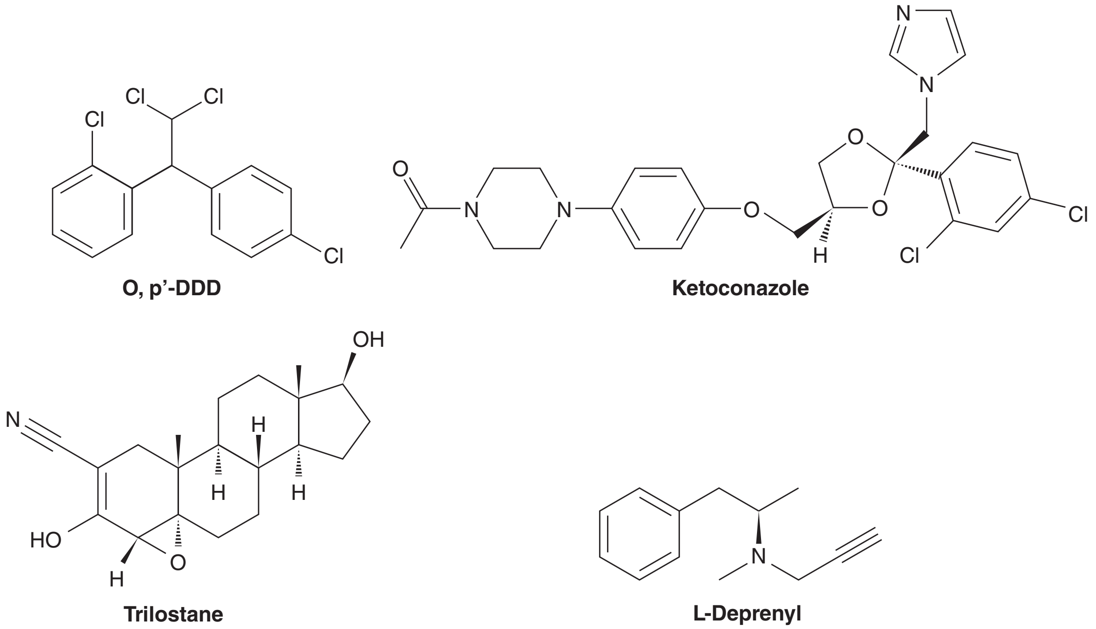


Figure 29.10 Structures of antiadrenal drugs.

Medical therapy for hyperadrenocorticism in the dog has been primarily aimed at reducing glucocorticoid production by the adrenal cortex. The two most frequently used drugs are mitotane and trilostane (Hoenig and Ferguson, 1991a; Neiger et al., 2002). Both drugs are used for hyperadrenocorticism regardless of etiology. A study has shown that the survival time of dogs treated with mitotane or trilostane was not significantly different (Arenas et al., 2014). Trilostane has also been used in horses (McGowan and Neiger, 2003). Hyperadrenocorticism rarely occurs in cats and the response to medical therapy has been mostly inconsistent. A study, however, indicates that trilostane was well tolerated and ameliorated signs of hyperadrenocorticism in a small number of cats (Mellett-Keith et al., 2013).

Mitotane

Chemistry and Mechanism of Action

Mitotane (1-(*o*-chlorophenyl)-1-(*p*-chlorophenyl)-2,2-dichlorethane; *o,p*'/4-DDD) (Figure 29.10) is a compound that is chemically similar to the insecticides DDD and DDT and leads to a relatively selective destruction of the zonae fasciculata and reticularis by an unknown mechanism.

Metabolism

Clinical studies in human patients indicate that approximately 40% of orally administered mitotane is absorbed, while the remainder is recovered in the feces. Similar studies are not available for the dog or cat.

Preparations and Properties

Mitotane (Lysodren; Bristol-Myers Oncology Division) is available in 500-mg scored tablets.

Therapeutic Uses

The cytotoxic effect of mitotane to the dog adrenal cortex was first described in 1959 (Villar and Tullner, 1959); however, it was not until 1973 that mitotane was used therapeutically in veterinary medicine (Lorenz et al., 1973; Schechter et al., 1973) and it became the most commonly used form of treatment for pituitary-dependent hyperadrenocorticism (Peterson, 1983). It has also proven to be efficacious in cases with adrenal neoplasia (Kintzer and Peterson, 1989; Feldman et al., 1992; Helm et al., 2011). Usually 25–50 mg/kg are given daily or divided twice daily for 7–10 days. The lower dose is usually given to dogs with concomitant disease, such as diabetes (see below in this section). The goal of this so-called loading period is to decrease the capacity of the adrenal cortex to the point that cortisol secretion becomes minimal and the animal is unable to respond to exogenous ACTH with an increase in cortisol secretion. If, after the loading period, the ACTH stimulation test indicates little or no response of the adrenal glands to ACTH and cortisol concentrations are low, the dog is kept on the same dose of mitotane, and a maintenance dose is given once weekly. To reduce side effects, it is advisable to divide the dose and give it over several days. If the ACTH stimulation test indicates a normal or even exaggerated response, the treatment with mitotane is continued and the dog retested at 5- to 10-day intervals until the desired response is obtained. In some cases, the mitotane dose needs to be increased to 75 mg/kg or 100 mg/kg to obtain the desired response. Higher loading doses are generally necessary to decrease cortisol concentrations in dogs with adrenal neoplasia and are followed by higher maintenance doses as well. Cortisol-secreting tumors seem to be more resistant and sometimes even unresponsive to the adrenolytic effect of mitotane.

Diabetic animals need to be monitored carefully while on mitotane treatment because the decrease in cortisol concentrations makes the animal more sensitive to insulin. For that reason, some clinicians prefer to use 25–35 mg/kg of mitotane instead of 50 mg/kg in the diabetic. Dogs on maintenance treatment need to be reevaluated regularly. An ACTH stimulation test is the most accurate assessment of adrenal functional reserve. If the ACTH test shows normal or exaggerated cortisol response, the animal should undergo “loading” again for several days, with an increase in the maintenance dosage. Cushingoid cats have shown an inconsistent response to mitotane treatment (Hoenig and Ferguson, 1991a; Kintzer and Peterson, 1989; Schechter et al., 1973; Valentin et al., 2014).

Side Effects

The side effects of mitotane at routine therapeutic doses are usually mild and may consist of gastrointestinal problems such as vomiting and anorexia, mild hypoglycemia, CNS depression, and mild liver damage with increases in alkaline phosphatase. However, in some cases the rapid fall in cortisol levels may lead to weakness, diarrhea, and lethargy. In rare cases, the zona glomerulosa is affected by mitotane, and electrolyte abnormalities compatible with Addison’s disease are seen (Kintzer and Peterson, 1989; Schechter et al., 1973).

Trilostane

Chemistry and Mechanism of Action

Trilostane (4α,5α-Epoxy-17β-hydroxy-3-oxoandrostane-2α-carbonitrile) produces suppression of the adrenal cortex by reversibly inhibiting enzymatic conversion of steroids by 3-β-hydroxysteroid dehydrogenase/δ-5,4 ketosteroid isomerase, thus blocking synthesis of adrenal steroids, including cortisol and aldosterone. The effect on aldosterone is less than that on cortisol (Wenger et al., 2004).

Metabolism

Trilostane is metabolized by the liver in people. There are no reports to date that describe the metabolism in dogs, cats, or horses.

Preparation and Properties

Trilostane (Vetoryl®, Dechra) is available in 30, 60 and 120-mg capsules. It should be stored at room temperature in airtight, light-resistant containers. Pregnant women should wear gloves when handling the drug (trilostane has been shown to cause abortion in pregnant monkeys and all users should wash their hands after handling the capsules).

Therapeutic Uses

Trilostane has been used for treatment of hyperadrenocorticism (pituitary dependent and adrenal dependent) in dogs (Neiger et al., 2002; Ruckstuhl et al., 2002; Wenger et al., 2004; Arenas et al., 2014), cats (Neiger et al., 2004; Valentin et al., 2014), and horses (McGowan and Neiger, 2003). In dogs, trilostane has been effective in reducing polydipsia/polyuria in over 90%, and polyphagia in over 80%. Trilostane causes a significant reduction in pre- and post-ACTH cortisol concentrations. It also leads to a significant decrease in alanine aminotransferase and alkaline phosphatase. Potassium concentrations increase but not usually to a level that requires medical intervention. Similarly, in horses, trilostane caused a reduction in polyuria/polydipsia and recurrent or chronic laminitis.

Trilostane is administered once daily at 2–10 mg per kg in dogs. Because accurate dosing with capsules is difficult, miniature dogs (<5 kg) usually receive 30 mg, small dogs (<20 kg) receive 60 mg; medium size dogs (<40 kg) 120 mg, and large dogs 240 mg. This needs to be adjusted according to clinical signs and cortisol values. The goal of therapy is to decrease post-ACTH cortisol concentrations measured 4–6 hours after administration of trilostane to 27–55 nmol/l (1–2 μg/dl).Some dogs may need twice-daily administration for control (Bell et al., 2006).

Cats have been treated successfully with an oral dose of 0.5–12 mg/kg every 12 or 24 hours, indicating a wide range in individual efficacy of the drug (Valentin et al., 2014). Others have suggested a mean initial dose of trilostane of 4.3 mg/kg if given once daily and 3.3 mg/kg if given twice daily (Mellett Keith et al., 2013). The dose in horses is 0.4–1.0 mg/kg once daily.

Side Effects

Trilostane seems to be generally well tolerated. Diarrhea, vomiting, and lethargy have been described, which are usually mild and self-limiting. However, acute deaths have also been seen in a small number of dogs (Neiger et al., 2002), and necrosis of the adrenal cortex (Chapman et al., 2004; Reusch et al., 2007) has been described. Interestingly, ultrasonographically, adrenal glands increase in size in dogs on trilostane (Mantis et al., 2003). This increase may be caused by the trophic effect of higher ACTH concentrations, which are due to lower negative feedback. Trilostane has been approved by the FDA for use in dogs in 2008.

Ketoconazole

Chemistry and Mechanism of Action

Ketoconazole (*cis*-1-acetyl-4-[4-[[2-(2,44-dichloro phenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl] methoxyl]phenyl]piperazine) (Figure 29.10) is an imidazole derivative whose major effect is to inhibit sterol synthesis in fungi (Schechter et al., 1973). In mammalian cells, it inhibits the conversion of lanosterol to cholesterol by inhibition of cytochrome P450–dependent enzyme systems (Loose et al., 1983). Ketoconazole also inhibits the synthesis of hormones from cholesterol such as cortisol, estradiol, and testosterone (Pont et al., 1940, 1982; Willard et al., 1986). In male dogs it greatly increases serum progesterone concentrations (Willard, 1989). Ketoconazole is a widely used antifungal agent in veterinary medicine and is fully discussed in Chapter 38.

Preparations and Properties

Ketoconazole (Nizoral®, Jannsen-Cilag) is available in tablet form, each containing 200 mg ketoconazole base, for oral administration. It is soluble in acids.

Metabolism

Ketoconazole requires an acidic environment for the dissolution of the drug. Its bioavailability is therefore decreased in patients on antacids. After oral ketoconazole administration, maximal blood concentrations occur 1–2 hours later. There is considerable variation in the bioavailability of the drug in dogs (Baxter et al., 1986). Approximately 50% of ketoconazole is excreted unchanged in the feces, and the rest is metabolized mainly by the liver. Inactive metabolites are excreted primarily in the feces; a small amount is excreted in the urine.

Therapeutic Uses

Ketoconazole is used to decrease cortisol concentrations in dogs with pituitary-dependent hyperadrenocorticism and cortisol-secreting adrenal neoplasms. The drug is expensive but in the past, before trilostane became available, it was a valuable alternative in those cases where mitotane was ineffective. It was also used as initial therapy prior to adrenalectomy in order to control the hyperadrenocorticism and reduce the risk of anesthesia and surgery. However, about 25% of dogs will not respond to this therapy. The recommended dose is 15 mg/kg twice daily. Because ketoconazole inhibits cortisol synthesis only reversibly, it should be given on a daily basis.

No consistent therapeutic effect of ketoconazole has been demonstrated in the cushingoid cat. The dose in the cat is 10 mg/kg twice daily.

Side Effects

The main side effects are vomiting and anorexia. Hepatic enzymes can transiently increase. In rare cases, a reversible hepatopathy with icterus may be seen (Willard, 1989). Gynecomastia and azoospermia have been reported in human patients (DeFelice et al., 1981). Ketoconazole is teratogenic and should not be used in pregnant animals.

L-Deprenyl

Chemistry and Mechanism of Action

L-Deprenyl hydrochloride (selegiline hydrochloride) is phenylisopropyl-*N*-methylpropinylamine (Figure 29.10) and is an irreversible inhibitor of monoamine oxidase B. L-Deprenyl is thought to decrease the metabolism of dopamine and also other catecholamines, to inhibit the reuptake of dopamine, and to increase its synthesis. It is hypothesized that the increase in dopamine concentrations decreases ACTH secretion from the pituitary and in turn cortisol secretion from the adrenal gland. In a study by Milgram and coworkers (1995), however, brain levels of dopamine were unaffected when dogs were given L-deprenyl for 3 weeks at different doses. The authors concluded that although dopamine was not increased, L-deprenyl could still affect dopaminergic transmission through one of its metabolites, phenylethylamine, whose levels were increased in dogs on L-deprenyl.

Preparations and Properties

L-Deprenyl (Anipryl®, Zoetis) is available for oral administration as white, convex tablets containing 2, 5, 10, 15, or 30 mg.

Metabolism

The metabolism of L-deprenyl in the dog is unknown. In humans, L-deprenyl is metabolized to amphetamine and metamphetamine; amphetamine concentrations also were increased in dogs on L-deprenyl (Reynolds et al., 1978). Pharmacokinetic analysis in four dogs indicated that the drug had a short half-life, which was attributed to high clearance and large volume of distribution of the drug. After oral administration, the bioavailability of the drug was less than 10% (Mahmood et al., 1994).

Therapeutic Uses

L-Deprenyl is rarely used anymore for the treatment of uncomplicated Cushing's disease and its primary indication is for the treatment of cognitive dysfunction. In 125 cases with naturally occurring pituitary-dependent hyperadrenocorticism, L-deprenyl was shown to be effective in controlling clinical signs associated with the disease such as panting, polyuria, polydipsia, obesity, reduced activity, abdominal distention, and others. The initial dose is 1 mg/kg body weight once daily; this dose might have to be increased if a response is not seen after 4 weeks. The maximum dose is 2 mg/kg body weight per day. It is recommended that the drug be administered for a 2- to 3-month period to allow sufficient time to assess its clinical usefulness. Should the dog's condition deteriorate during that time or should the dog show complications due to high cortisol levels, L-deprenyl should be discontinued and treatment with mitotane initiated. Although the company claims that 80% of dogs with hyperadrenocorticism respond favorably to L-deprenyl treatment, a study in a very small group of dogs suggests that only about 20% show an improvement of the clinical signs associated with the disease (Reusch et al., 1999). It has been argued that only cushingoid dogs with pituitary pars intermedia tumors might respond to this treatment. Pars intermedia tumors account for approximately 30% of pituitary tumors in dogs with Cushing's disease (Peterson, 1999).

Side Effects

L-Deprenyl is a very safe drug. The following adverse effects were noted infrequently in dogs on long-term treatment: vomiting, diarrhea, hyperactivity, anorexia, diminished hearing, weight loss, anemia, polydipsia, and weakness.

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30

Drugs Influencing Glucose Metabolism

Margarethe Hoenig

Insulin

History

Diabetes mellitus has been recognized for centuries as a debilitating disease, characterized by the excretion of "sweet urine" (mellituria), polydipsia, wasting of tissue, and the development of ketoacidosis, hyperosmolar coma, and death. The role of the pancreas in the development of diabetes was first recognized in 1886 when Minkowski and von Mering produced diabetes by total pancreatectomy in the dog. In 1921, Banting and Best extracted the active compound from pancreas and showed that it was able to control hyperglycemia in diabetic dogs and humans. Insulin was prepared for the first time in crystalline form in 1926 by Abel, and it took 34 more years until its amino acid sequence was demonstrated by Sanger. Finally, in 1963 insulin was synthesized by Meyenhofer and coworkers, and in 1964 by Katsoyannis and coworkers. In 1967, Steiner discovered that insulin was synthesized as a larger precursor, proinsulin. An even larger molecule, named preproinsulin, was identified as a precursor for proinsulin in 1976 by Chan and coworkers (for a detailed review see Bliss, 1983). These research efforts have culminated in the biosynthesis of human insulin by recombinant DNA techniques, allowing large-scale production of the hormone in 1983 (Frank and Chance, 1983; Johnson, 1983).

Spontaneous diabetes has been reported in many species. Diabetes is a major health problem and one of the leading causes of death in humans. In animals, it occurs most frequently in the dog and cat, with an incidence of approximately 0.5–1%. Overt diabetes is rare in horses, cattle, and sheep, although obese horses may develop metabolic syndrome, which might include abnormal glucose concentrations. Isolated cases of diabetes have been reported in mules, ferrets, pigs, newts, buffalos, monkeys, and fish (Stogdale, 1986). Diabetes also has been reported in several breeds of birds (Stogdale, 1986). Diabetes in humans has been classified into two major categories, which are of interest to veterinary medicine: type 1 diabetes, previously called insulin-dependent diabetes mellitus or juvenile-onset diabetes, and type 2 diabetes, previously called noninsulin-dependent diabetes mellitus or maturity-onset diabetes. Based on glucose-tolerance testing (Kaneko et al., 1977; O'Brien et al., 1985), it has been determined that most diabetic dogs and cats are insulin deficient.

Chemistry and Biosynthesis

Insulin is produced in the beta cells of the islets of Langerhans in the endocrine pancreas. The islet also contains glucagon-secreting alpha cells, somatostatin-secreting delta cells and PP or F cells, which secrete pancreatic polypeptide. The beta cells compose about 60–80% of the islet. The endocrine cells are arranged in a nonrandom distribution, with beta cells forming a central core surrounded by a mantle of the other three cell types in some animals and humans (Bonner-Weir and Orci, 1982). In the cat, the beta cells are located in the periphery (O'Brien et al., 1986), while in the horse, the alpha cells form a central core (Helmstaedter et al., 1976) surrounded by beta cells.

Insulin exists initially as preproinsulin, which is cleaved to proinsulin in the endoplasmic reticulum. Proinsulin is a large polypeptide consisting of an A and B chain and a connecting peptide (Figure 30.1). By proteolytic cleavage, four basic amino acids and the connecting peptide are removed, and proinsulin is converted to insulin (Steiner et al., 1969). This reduces the molecular weight from 9000 (proinsulin) to one of 6000 (insulin) and one of 3000 (C-peptide). This conversion occurs soon after proinsulin is transported to the Golgi complex, where it is packaged into secretory granules. These granules therefore contain an equimolar amount of insulin and C-peptide. In the secretory granules, insulin is complexed with zinc and stored.

The amino acid sequence of insulin shows little species variation. Human insulin differs from porcine and rabbit insulin by a single amino acid, whereas dog insulin is identical to porcine insulin. Bovine insulin differs from

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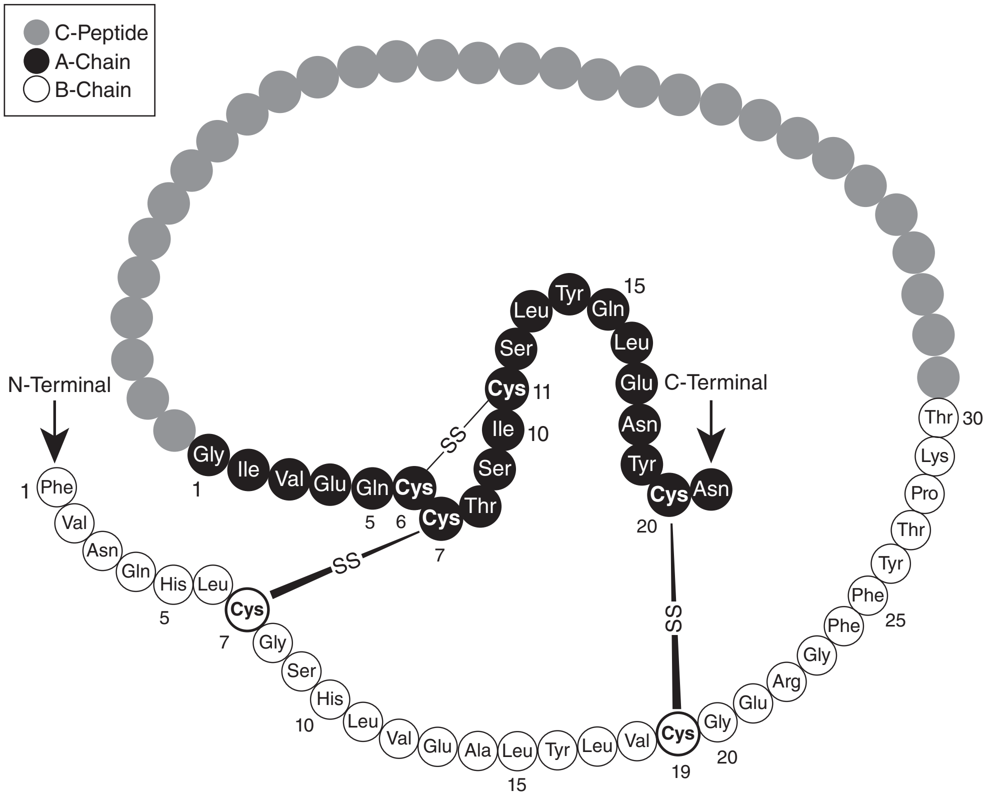


Figure 30.1 Proinsulin.

human insulin by three amino acids and from feline insulin by one amino acid (Neubauer and Schoene, 1978; Porte and Halter, 1981; Hoenig et al., 2006a). Antiserum directed against insulin of one species usually cross-reacts with insulin of other species. Antiinsulin serum generally also cross-reacts with heterologous proinsulin, while C-peptide has no immunoreactivity against insulin antiserum. The biological activity of proinsulin, however, is much less than that of insulin. Feline proinsulin has been cloned and expressed (Hoenig et al., 2006a).

Secretion

The secretion of insulin from the beta cell occurs through the process of exocytosis in which there is fusion of the secretory granule with the plasma membrane. Insulin secretion is highly regulated primarily by glucose, but also by other fuels, hormones, and neurotransmitters (Porte and Halter, 1981). On a cellular level, insulin release stimulated by glucose and other fuels is thought to be initiated by closure of ATP (adenosine triphosphate)-dependent K channels, which leads to depolarization of the beta cell and influx of calcium into the cytosol through voltage-gated calcium channels (Arkhammar et al., 1987; Cook et al., 1988). The increase in cytosolic free calcium triggers pulsatile insulin secretion. In addition, augmentation of this response occurs by both a K^+-ATP channel-independent Ca^{2+}-dependent pathway and K^+-ATP channel-independent Ca^{2+}-independent pathways of glucose action (Henquin, 2011). The pathway that is Ca^{2+} dependent but independent of ATP-K channels is called the amplification pathway. The exact mechanism is not understood (for review see Straub and Sharp, 2002). Insulin is released from the beta cell in a pulsatile fashion. It is thought that these rapid oscillations are generated through intraislet mechanisms, because they can be seen even in isolated islets and beta cells (Berman et al., 1993). Pulsatile secretion becomes abnormal in diabetes; however, pulsatility can be restored by beta cell rest (Laedtke et al., 2000).

In both type 1 and type 2 diabetes, islet function and insulin secretion are abnormal. It is now clear that in both types a loss of beta-cell mass has occurred and that the fundamental difference between the two major types of diabetes lies in the etiology of the beta-cell dysfunction and its severity but not its presence or absence (Porte, 1991). It is controversial whether a reduction in beta cell mass or function play the major role in the pathogenesis of type 2 diabetes (for review see Meier and Bonadonna, 2013). It appears that both occur together and are tightly related. Insulin resistance, frequently seen with obesity in humans and cats (Kahn et al., 2006; Hoenig et al., 2006b) is an important concomitant factor in type 2 diabetes and puts an additional stress on the beta cell. However, the insulin resistance of obesity alone is not sufficient to cause diabetes. This is shown by the fact that over 80% of obese people are not diabetic; a similar assessment has not been made in cats. It is not known how many obese cats actually become diabetic. Obesity increases the risk of diabetes; however, most obese cats have normal glucose control because they counteract peripheral insulin resistance with a decrease in endogenous glucose output by the liver (Kley et al., 2009; Hoenig et al., 2011, 2012, 2013). It is interesting that, while the prevalence of obesity in people and cats is about equal, about 8% of humans develop type 2 diabetes, whereas the prevalence of diabetes in cats is <1%. The reason for this discrepancy is not known.

Evidence suggests that disordered insulin secretion may contribute to development of insulin resistance and hence represent an initiating factor in the progression to type 2 diabetes (Schofield and Sutherland, 2012). An early marker of beta cell dysfunction is a loss of pulsatility of insulin secretion. This leads to hepatic insulin resistance and higher uncleaved proinsulin concentrations (for review see Wahren et al., 2012). An early marker is also a change in the proinsulin/insulin secretion ratio (Haffner et al., 1997). In cats and humans, among a few others, the increase in insulin release from a reduced beta-cell mass in type 2 diabetes is associated with release and deposition of islet amyloid polypeptide, leading to further loss of beta cells (Porte and Kahn, 1989; Hoenig et al., 2000a). It is thought that the majority of dogs have a form of diabetes similar to type 1 diabetes in people, that is involving a genetic component, which is influenced by environmental input. The possibility of a genetic basis for canine diabetes has been suggested by the occurrence of the disease in dog families, and the presence of DLA haplotypes that seem to confer risk of diabetes. In type 1 diabetes in people, the loss of beta cells is caused primarily by immune-mediated destruction (MacLaren et al., 1989). Beta-cell antibodies are present in approximately 50% of naïve diabetic dogs (Hoenig and Dawe, 1992). In later studies, 4/30 dogs were found to be positive for antibodies against the 65-kDa isoform of canine glutamic acid decarboxylase and 3/30 also had antibodies against the insulinoma-associated antigen-2 (IA-2), both frequently found in human type 1 diabetics (Davison et al., 2008a). In another study, 5/40 newly diagnosed dogs had antibodies against insulin (Davison et al., 2008b). Beta-cell antibodies have not been demonstrated in diabetic cats (Hoenig et al., 2000b).

Insulin secretion can also become autonomous, resulting in hyperinsulinemia associated with hypoglycemia. Insulin-secreting tumors (adenomas or carcinomas; also called insulinomas) occur infrequently and have been described in the dog, cat, ferret, cattle, and a pony (Capen, 1990). The diagnosis of an insulinoma can be difficult, but frequently it can be made on the basis of an inappropriately high serum insulin concentration. In humans, the diagnosis is often made based on high proinsulin and/or C-peptide concentrations (Chammas et al., 2003).

Mechanism of Action

Insulin is the most potent physiological anabolic agent. In insulin-responsive tissues, it facilitates cellular uptake and metabolism of glucose. Insulin promotes the synthesis of glycogen, protein, and fat and is involved in the uptake of ions such as K into the cell. Insulin exerts its effect by binding to specific receptors on the plasma membrane of cells. The insulin receptor is a tetrameric structure consisting of two subunits: an alpha and a beta subunit. Two of each of these subunits are joined together via disulfide bonds. Both subunits are glycosylated and exposed to the extracellular milieu; only the beta subunit is exposed to the intracellular environment. The insulin receptor has tyrosine kinase activity. Binding of insulin leads to autophosphorylation of the receptor and these phosphorylated Tyr residues serve as docking sites for downstream effectors (Le Roith and Zick, 2001; Schinner et al., 2005). Once the insulin receptor kinase is activated, the presence of insulin is not necessary for its continued activity. The receptor is inactivated by dephosphorylation.

Despite our knowledge of insulin–receptor interaction, our understanding of molecular events that link the insulin–receptor to the regulation of cellular metabolism is poor. Some, but not all, of the actions are due to the phosphorylation or dephosphorylation of target proteins on serine and threonine residues. The mediation of the mitogenic responses involves the mitogen-activated protein (MAP) kinase pathway, whereas the metabolic responses of insulin, such as glucose transport, involve phosphatidyl-inositol-3 (PI3)-kinase. Diabetes has also been linked to alterations in the function of both endoplasmic reticulum (Kaneto et al., 2005) as well as mitochondria (Lowell and Shulman, 2005).

Metabolism

The existence of a relatively specific insulin-degrading enzyme (IDE, insulinase, insulin-specific protease) has been reported by several investigators (for a review see Duckworth, 1990). Insulin-degrading activity has been found in essentially all tissues examined, and high activity has been seen in liver, kidney, muscle, brain, fibroblasts, and red blood cells. The enzyme seems to be located predominantly in the cytoplasm but can be found in other subcellular compartments. Plasma membrane preparations also contain this enzyme. Some receptor-bound insulin is degraded on the membrane, while some insulin is released intact from the receptor, and some is

Table 30.1 Insulin preparations

Trademark	Common name	Strength	Administration	Company
Humalog®	Lispro	U100	External pump, Intravenous, subcutaneous	Eli Lily
Novolog®	Aspart	U100		Novo Nordisk
Apidra®	Glulisine	U100		Sanofi-Aventis
Humulin® R	Regular human insulin, recombinant DNA origin	U100	External pump, intravenous intramuscular subcutaneous	Eli Lily
Humulin® R		U 500		
Humulin® N	NPH human insulin, recombinant DNA origin	U100	Subcutaneous	
Prozinc®	Protamine zinc human recombinant insulin, DNA origin	U40		Boehringer-Ingelheim
Vetsulin®	Pork zinc insulin	U40		Merck Animal Health
Lantus® (Glargine)	Recombinant human insulin analog, DNA origin	U100		Sanofi-Aventis
Levemir® (Detemir) Detemir	Recombinant human insulin analog, DNA origin	U100		Novo Nordisk
Tresiba® (Insulin Degludec)	Recombinant human insulin analog, DNA origin	U200		

internalized with the receptor, and insulin degradation is then initiated in endosomes. The degradation of insulin via this process is rapid. Other enzymes may also be involved in the degradation of insulin, and their role and factors controlling insulin-degrading activity in various tissues and disease states needs to be examined.

Preparations and Properties

The biological activity of insulin preparations is documented using a bioassay that is based on the capacity of insulin to lower blood glucose concentrations. All but one insulin preparation used in human medicine have been standardized at 100 U/ml; a U 500 (500 U/ml) regular insulin preparation is still available from Eli Lilly. All insulin preparations used for humans are of recombinant origin, either containing the exact amino acid sequence of human insulin, or mutations thereof. This has led to a variety of new insulins with different action times. Regular human insulin, lispro, glulisine, and aspart can be given intravenously. There are two insulins that have been approved for veterinary use: ProZinc® is a human recombinant protamine zinc insulin, and Vetsulin® is a pork zinc insulin and is currently the only animal-derived insulin still available on the US market. Both are approved for use in cats; Vetsulin in addition is for use in dogs. Both of these insulins contain 40 U/ml and are administered subcutaneously. For correct dosage, insulin preparations are administered with syringes that are calibrated for the appropriate standardization (e.g., U 100 syringes, U 40 syringes). All commercially available insulins in the United States contain no more than 25 parts per million of proinsulin. The insulin preparations currently available are summarized in Table 30.1.

Based on action profiles, insulin preparations can be divided into fast, intermediate, and long acting. Generally, lispro and aspart, as well as the regular insulin preparations, have a quick onset of action (15-30 minutes) and short duration, whereas the NPH (neutral protamine Hagedorn), glargine, detemir, protamine zinc, and the pork zinc insulin preparations have a longer onset and duration of action. There is, however, considerable species and individual variation in the insulin action profiles. It is important for the practitioner to realize that the time course of action of any insulin preparation may not only vary considerably between different individuals, but also in the same individual from day to day (Hoenig and Ferguson, 1991; Church, 1981). It is also important to realize that the pharmacokinetics change with different doses. The time periods listed by manufacturers should only be considered as initial guidelines. Individual responses to insulin and the “fine-tuning” of insulin therapy have to be assessed by measuring blood glucose concentrations at frequent intervals, yielding what is called a “glucose curve”. In order to decrease stress effects, glucose monitoring also can be performed by measuring interstitial glucose concentrations using a continuous glucose monitoring system (e.g., Guardian Real-Time; Medtronic). Of the newer short-acting insulin preparations, lispro has been used successfully for the treatment of ketoacidotic dogs (Sears et al., 2012). There are currently no reports of the clinical use of aspart in pets. The pharmacokinetics of insulin aspart are similar to those of regular insulin when given intravenously in people. The plasma half-life is 11 minutes with insulin aspart and 12 minutes with regular insulin. When given subcutaneously, there is also little difference between the two insulins. However, both show great individual variations in plasma concentrations (Plum et al., 2000).

Glargine and detemir have been used extensively in diabetic cats (Weaver et al., 2006; Roomp and Rand, 2009; Roomp and Rand, 2012) and also in dogs (Sako et al., 2011; Fracassi et al., 2012; Hess and Drobatz, 2013). Although labeled as "long-acting" insulin preparations, they usually need to be given twice daily for optimal glucose control. The dosage of detemir is generally less than that of other insulin preparations.

Insulin, thus far, needs to be injected for the effective treatment of diabetes. The short-acting insulin preparations can be injected intravenously, intramuscularly, and subcutaneously; the intermediate and long-acting insulin preparations are for subcutaneous injection. The preferred injection site is the flank for those injections. Some of the intermediate and long-acting insulin preparations, but not detemir and glargine, are suspensions and need to be gently mixed before administration. It is recommended to shake pork zinc insulin vigorously before the first use. The shelf life of most insulin preparations is 28 days when stored at room temperature. If refrigerated, unopened bottles last until the expiration date of the vial.

Attempts have been ongoing to produce insulins that do not require injection. Exubera®, which was produced by Pfizer, Sanofi-Aventis, and Nektar Therapeutics, was a rapid-acting, dry-powder type of insulin that was delivered to the lungs via a special bong-shaped inhaler device, and received FDA approval in 2006. It was withdrawn from the market in 2007 because of weak sales. Oral-lyn® has been developed by Generex. It is a novel oral (buccal) insulin spray product, which is delivered into the human mouth by way of the company's proprietary RapidMist drug delivery system. It completed phase III clinical trials in India in 2013 and is awaiting approval by the Indian authorities. It remains to be seen if such a preparation could be used in pets.

Therapeutic Uses

Treatment of Diabetes Mellitus in Dogs and Cats

Insulin deficiency is characterized by increased catabolism. The breakdown of glycogen, increased endogenous glucose output, and decreased utilization of glucose in peripheral tissues, especially muscle and adipose tissue, leads to hyperglycemia. Protein breakdown fuels gluconeogenesis, and fat breakdown leads to increased fatty acids in blood and their transfer primarily to the liver, where they are used for energy, ketone body or very low-density lipoprotein (VLDL) production. They can also be reesterified leading to fatty liver.

Treatment of diabetes must be adjusted to the clinical presentation of the animal. Usually, two broad forms can be differentiated: uncomplicated and complicated diabetes mellitus. For practical purposes, complicated diabetics are those that cannot take food orally, such as the vomiting ketoacidotic patient or the comatose hyperosmolar patient (Hoenig and Ferguson, 1991).

The goal of treatment of a diabetic dog is to maintain blood glucose concentrations in a high normal to mildly hyperglycemic range for most of the day to alleviate the biochemical effects and clinical signs of insulin deficiency and avoid hypoglycemia. In cats, the treatment regimens are controversial. Some clinicians advise to maintain blood glucose concentrations in a normal to mild hyperglycemic state, similar to what is recommended for diabetic dogs; that is, the blood glucose should stay between 5 and 15 mmol/l (90-270 mg/dl) for most of the day (Reusch, 2010), and the nadir should be between 90 mg/dl and 160 mg/dl, avoiding the dangers of hypoglycemia in a patient that cannot convey the more subtle signs of low blood glucose. Others have proposed an insulin treatment regimen even more rigorous than what is desirable in humans (Roomp and Rand, 2012), and advocated to achieve glucose concentrations in diabetic cats between 2.8 and 5.5 mmol/l (50 and 100 mg/dl), that is a range where cats may experience biochemical as well as clinical hypoglycemia. The goal of this regimen is to increase remission rates. While these investigators have reported higher remission rates than others, without blinding the studies and providing detailed information about the health status of the patients entering those studies and the feeding protocols, a comparison between the success of a particular treatment regimen is not possible. Furthermore, the dangers of hypoglycemia cannot be ignored and might compromise the quality of life of both owner and pet.

The fast-acting insulin preparations are used for the complicated diabetic patient. These animals can be treated with low-dose constant rate insulin infusions (see Table 30.2 for treatment protocol) or intramuscular insulin injections with regular insulin or lispro insulin using 0.1 units/kg as needed to keep blood glucose concentrations at approximately 200 mg/dl. Glargine, a long-acting insulin preparation, has also been used for the treatment of ketoacidosis in cat and was given intramuscularly as well as subcutaneously (Marshall et al., 2013). Because of glargine's long duration; however, such a protocol would eliminate the ability to quickly intervene.

The intermediate and long-acting insulin preparations are used in the diabetic that is able to eat and drink without vomiting. As stated above, it is important to realize that each animal reacts differently to a given insulin preparation, which makes glucose control challenging

Table 30.2 Low-dose, continuous IV infusion protocol for the treatment of diabetic ketoacidosis. Source: Modified from Macintire, 1993 and Sears et al., 2012.

1. Place intravenous catheter.
2. Start fluid therapy (0.9% NaCl or Normosol R; consider replacement and maintenance fluid requirements, as well as supplementation with K and/or P as needed). Rehydrate the animal.
3. [Insert urinary catheter and attach to a closed monitoring system.]
4. Add regular insulin or lispro insulin (2.2 units/kg/day) to 250 ml of saline solution. Use pediatric infusion set or infusion pump for accurate administration. Use protocol shown below.
5. Monitor blood glucose concentrations every 1 hour.
6. Monitor urinary output.
7. Monitor electrolytes every 8 hours. Adjust if necessary.
8. Change saline solution to 2.5% dextrose in 0.45% saline, when blood glucose reaches approximately 250 mg/dl (13.8 mmol/l). Continue to administer insulin. Supplement K and/or P as needed. Adjust fluid rate and glucose concentration of the fluid as follows:

Blood glucose (mg/dl)	IV fluids	Insulin rate (ml/hour)
>250	0.9% saline	10
200–250	0.9% saline 2.5% dextrose	7
150–200	0.9% saline 2.5% dextrose	5
100–150	0.9% saline 5% dextrose	5
<100	0.9% saline 5% dextrose	stop

9. Keep animals on this regimen until they are able to eat without vomiting.
10. Stop insulin infusion in the evening. Start treatment of the uncomplicated diabetic the next morning.

for the veterinarian. However, most animals need twice-daily insulin administrations for good control, regardless of the fact that a so-called intermediate or long-acting insulin preparation has been used. It is prudent to maintain a treatment regimen for several days before trying to adjust the time and dose of insulin or the time of feeding, unless insulin leads to hypoglycemia. It may take several weeks before an animal shows a consistent response to a given preparation. Hypoglycemic episodes make it more difficult to regulate a diabetic because of the insulin-antagonistic effects of counterregulatory hormones, which are secreted in response to low blood glucose concentrations (called Somogyi rebound).

Treatment of Ketosis in Cattle

Insulin is a powerful antiketogenic agent and has been used as adjunct treatment in ketosis therapy. It has been suggested that insulin therapy is particularly beneficial in those cases of ketosis that occur within the first week of lactation and are nonresponsive to glucose or glucocorticoid therapy alone (Herdt and Emery, 1992). A dose of 200–300 IU of protamine zinc insulin per animal, repeated as necessary at 24 to 48-hour intervals, was administered.

Adverse Effects

Acute hypoglycemia may result from excessive insulin dose, especially in the presence of inadequate food intake. The brain is particularly sensitive to glucose deficiency, and nervous system dysfunction is seen. Initially, confusion, nervousness, trembling, or hyperexcitability may be seen, which progress to convulsions if the hypoglycemia is not treated. Karo® syrup or other glucose-containing solutions can be used orally; however, the intravenous administration of dextrose solutions may be necessary to alleviate the hypoglycemic symptoms. Glucagon may also be used intramuscularly or as a constant rate infusion (see Section Glucagon; Fischer et al., 2000).

The body itself combats hypoglycemia through the release of insulin-antagonistic hormones, primarily catecholamines, glucagon, glucocorticoids, and growth hormone. This frequently leads to hypoglycemia-induced hyperglycemia, the so-called Somogyi rebound, mentioned in Section Treatment of Diabetes Mellitus in Dogs and Cats (Cryer and Gerich, 1990).

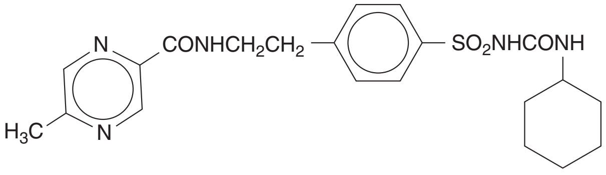
Antibody formation against insulin has been documented in diabetic dogs (Davison et al., 2003) and diabetic cats (Hoenig et al., 2000b). While this has not been examined in dogs, in cats the insulin dose was not different in the presence or absence of antibodies, and the clinical significance therefore seems to be minor.

Oral Hypoglycemic Agents

Sulfonylureas

History

During World War II, Janbon and colleagues, in 1942, found that certain sulfonamide derivatives being used to treat typhoid fever caused hypoglycemia. After the end of the war, derivatives of these antibiotics were first used to treat patients with diabetes. Studies by Loubatieres showed that sulfonylureas did not alter blood glucose concentrations in pancreatectomized dogs and juvenile diabetics (i.e., type 1), which suggested that sulfonylureas



Glipizide

Figure 30.2 Chemical structure of the sulfonylurea glipizide.

stimulated the pancreas to secrete insulin (Loubatieres, 1946). Indeed, sulfonylureas have become the major therapeutic agent used to treat type 2 diabetes in humans until recently. In type 2 diabetics insulin secretion is still present, at least initially, although insufficient to control blood glucose concentrations. In most diabetic dogs and cats the insulin-secretory capacity of the beta cells is lost, and they require insulin for treatment.

Chemistry

The chemical structure of the most commonly used sulfonylurea in veterinary medicine, glipizide, is shown in Figure 30.2. Oral sulfonylurea agents available in the United States are listed in Table 30.3. Sulfonylureas differ in potency, duration of action, metabolism, and side effects.

Mechanism of action

In the pancreatic beta cells, sulfonylureas inhibit ATP-dependent potassium (K^+) channels in the plasma membrane. This results in depolarization and release of insulin (Antomarchi et al., 1987). Because ATP-sensitive K^+ channels also exist in other tissues, sulfonylureas exert tissue-specific responses through the activation of the channels. The extrapancreatic effects of sulfonylureas have been described in detail by Gerich (1989). Sulfonylurea therapy augments the ability of insulin to inhibit hepatic glucose production and to stimulate glucose utilization. It is unclear if these effects are direct effects of the drugs or secondary to the reduced hyperglycemia caused by improved insulin secretion.

Table 30.3 Oral sulfonylurea agents available in the United States

Tolbutamide: Orinase (Upjohn) and generics
Tolazamide: Tolinase (Upjohn) and generics
Chlorpropamide: Diabinese (Pfizer) and generics
Acetohexamide: Dymelor (Lilly) and generics
Glyburide: Micronase (Pfizer) and DiaBeta (Sanofi-Aventis)
Glipizide: Glucotrol (Pfizer)
Glimepiride: Amaryl (Sanofi Aventis) and generics

Metabolism

In people, sulfonylureas are readily absorbed from the gastrointestinal tract and are highly protein-bound. Glipizide has a half-life in plasma of approximately 2–4 hours, but its hypoglycemic effects may persist for up to 24 hours. It is metabolized in the liver, and its inactive metabolites are excreted in the urine (Gerich, 1989). Sulfonylureas should not be administered in patients with renal or hepatic insufficiency. Glipizde interacts with other drugs and its effectiveness might either be increased (e.g., with H_2 antagonists, anticoagulants, sulfonamides, salicylates) or decreased (e.g., thyroid medication, phenothiazines, corticosteroids, beta blockers).

No studies on the pharmacokinetics of glipizide have been done in the cat or dog. Studies in healthy cats showed an immediate release of insulin after oral administration. Peak insulin concentrations were achieved 15 minutes later, and baseline concentrations were reached again after 60 minutes (Miller et al., 1992). In our own studies, the insulin concentration peaked 30 minutes after glipizide administration and did not return to baseline levels until several hours later (Hoenig, unpublished). Glipizide is usually administered orally. A study has evaluated transdermal administration of glipizide. The absorption was low and inconsistent and it was concluded that a transdermal preparation cannot be recommended at this time (Bennett et al., 2005).

Preparations and properties

The preparation used most frequently in veterinary medicine is glipizide (Glucotrol®; Pfizer). It is marketed as 5 and 10-mg tablets.

Therapeutic uses

Glipizide has been advocated as an antidiabetic agent for the cat (Miller et al., 1992). The drug seemed to be effective in some cats and is given at a dose of 2.5–5 mg BID.

For glipizide to be effective, it is necessary for beta cells to still have the capacity to release insulin. Therefore, in theory, this drug should only be administered in those cases in which insulin secretion has been documented (with glucose/glucagon stimulation tests, etc.). However, it has been shown that glipizide accelerates amyloid deposition in cat islets (Hoenig et al., 2000a), which confirmed evidence in rats that these drugs may actually be harmful (Kahn et al., 1993). It is therefore prudent to treat all diabetic cats with insulin at this time (i.e., the insulin-deficient patient whose beta cells no longer can secrete insulin and the patient who still has insulin but whose secretion rate is insufficient to control blood glucose concentrations) until other oral antihypoglycemic agents have been evaluated and shown to be effective.

Adverse effects

Hypoglycemia is the most frequently observed side effect of sulfonylureas in humans; however, severe life-threatening hypoglycemic reactions are rare. The hypoglycemic reactions may be potentiated by drugs that are highly protein-bound. In cats, vomiting, hypoglycemia, icterus, and an increase in alanine aminotransferase (ALT) have been reported (Feldman et al., 1997). They were all reversible when the drug was either discontinued or the dosage decreased. Other side effects seen in humans include increased cardiovascular mortality, gastrointestinal disturbances, allergic skin reactions, and hematological changes (Gerich, 1989).

Metformin

Metformin (*N,N*-dimethylbiguanide; Glucophage®, Bristol-Myers Squibb; and generics) is an oral antihyperglycemic agent used primarily in the management of type 2 diabetes in people. Metformin reduces blood glucose concentrations primarily by reducing hepatic glucose output and by improving peripheral sensitivity to insulin without affecting insulin secretion. It does not cause hypoglycemia at therapeutic doses. Although the pharmacokinetic profile of metformin has been established in the cat (Michels et al., 1999) and is similar to that in people, data on the efficacy of the drug in clinical diabetes cases are sparse. When metformin was used in five diabetic cats (Nelson et al., 2004), only one showed a beneficial response after 8 weeks of treatment, three showed no response, and one cat died unexpectedly. The drug is eliminated primarily by renal clearance in the cat (Michels et al., 1999) and should not be used in cats with significant renal dysfunction.

Thiazolidinediones

Thiazolidinediones (TZDs, or "glitazones") are orally administered insulin sensitizers marketed for use in human type 2 diabetes. TZDs are agonists of the peroxisome proliferator activated receptor gamma (PPARγ) (Tontonoz and Spiegelman, 2008), a transcription factor belonging to the nuclear hormone receptor superfamily. PPARγ plays a key regulatory role in metabolism, cellular differentiation, and cell growth, and is crucial for adipocyte development, lipid transport and storage, and whole-body glucose homeostasis (Menendez-Gutierrez et al., 2012). For the TZDs, adipose tissue is likely to be a major site of action through direct PPARγ activation. PPARγ is abundant in mature adipocytes, and many more genes are differentially expressed in adipose tissue after TZD treatment than are affected in other tissues such as muscle and liver (Way et al., 2001; Rasouli et al., 2012). Proteins known to be upregulated in adipocytes by TZD treatment include glucose and fatty acid transporters, fatty acid binding and sequestration proteins, metabolic enzymes, and many others (Lefterova et al., 2010; Tontonoz and Spiegelman, 2008).

In addition to their benefits regarding glycemic control, TZDs improve indices of beta-cell function in human diabetes (Gastaldelli et al., 2007). Clinically, the consequences of beta-cell functional improvement and preservation by the TZDs are durable long-term glycemic control (DeFronzo, 2010) and reduction of diabetes risk in susceptible populations (DeFronzo et al., 2011). In a large cohort of humans with impaired glucose tolerance ("prediabetics"), pioglitazone reduced the risk of conversion to diabetes by 72% (DeFronzo et al., 2011).

Among the thiazolidinedione compounds, troglitazone (Rezulin®, Parke Davis) was the first to become widely available (Nolan et al., 1994). The serious liver side effects forced the discontinuation of the use of this drug. Pioglitazone (Actos®, Takeda) and rosiglitazone (Avandia®, GlaxoSmitKline) have been approved by the FDA in 1999 for the treatment of type 2 diabetics. Because of an increased incidence of cardiovascular adverse events, rosiglitazone was removed from the US market and placed on a restricted prescription list. Pioglitazone is now the only TZD available for general prescription. The pioglitazone pharmacokinetics have been evaluated in cats (Clark et al., 2012). It was found that it was rapidly absorbed after oral administration, with a mean bioavailability of 55%. Volume of distribution of the drug after IV administration was similar to that seen in other species, while clearance was increased and half-life decreased relative to humans. It was shown in a randomized placebo-controlled study that 3 mg/kg pioglitazone administered for 7 weeks significantly improved insulin sensitivity and lowered serum triglyceride and cholesterol concentrations in obese cats. No adverse effects attributable to pioglitazone were evident in the otherwise healthy obese cats at this dosage and duration (Clark et al., 2014). There are no reports about the efficacy of this drug in diabetic cats or in cats with lipid disorders.

Acarbose

Acarbose (Precose®, Bayer) is an alpha glucosidase inhibitor, a term applied to inhibitors of alpha amylase, which digests starch, and of brush border oligo- and disaccharidases, which cleave off glucose. Acarbose delays and reduces postprandial hyperglycemia after a carbohydrate meal. It has been used in human diabetic patients in combination with insulin and oral antihyperglycemic agents but also as monotherapy. Gastrointestinal disturbances such as diarrhea, flatulence, and abdominal pain are the major side effects (Scheen and Lefebvre, 1998). The pharmacokinetics of acarbose has been established in the dog (Ahr et al., 1989), and a small number of diabetic dogs needed less insulin when treated with acarbose

(Robertson et al., 1998). It has also been used in diabetic cats in combination with a low-carbohydrate diet (Mazzaferro et al., 2003).

Sodium–Glucose Cotransporter 2 Inhibitors

Sodium–glucose cotransporters (SGLTs) are a large family of membrane proteins that transport glucose, among other substances, across the brush-border membrane of the intestinal epithelium and proximal renal tubules (Ghezzi and Wright, 2012). Two SGLTs are most important in affecting glucose transport in the intestine and kidney. The low-capacity, high-affinity transporter SGLT1 is expressed mainly in the small intestine and late proximal tubules of the kidney, whereas the high-capacity, low-affinity transporter SGLT2 is found primarily in the early proximal tubules and is responsible for most of the glucose reabsorption (Kurosaki and Ogasawara, 2013). Inhibitors of SGLT2 have been investigated for their use in the treatment of diabetic hyperglycemia three decades ago. At that time it was shown that phlorizin, a nonselective SGLT inhibitor, could correct hyperglycemia and insulin resistance in diabetic rats (Rossetti et al., 1987). Today, several SGLT2 inhibitors (canagliflozin, empagliflozin, and dapagliflozin) are on the human market. They can be used alone but are also produced in combination with metformin or a DPP-4 inhibitor (see Section Glucagon-Like Peptide 1 and Dipeptidyl Peptidase 4 Inhibitors). They are currently approved for the treatment of type 2 diabetes in people, resulting in an increased amount of glucose being excreted in urine. They lower HbA1c without increasing the risk of hypoglycemia, and they contribute to reductions in body weight and blood pressure (Mudaliar et al., 2015). Side effects in humans include genitourinary infections and ketoacidosis, although the latter appears to be rare. There are currently no published reports about their use in diabetic pets.

Glucagon

History

Although the existence of a pancreatic hyperglycemic hormone was postulated in 1923 by Kimball and Murlin (1923), it was not until 1955 that Staub and coworkers succeeded in purifying glucagon (Staub et al., 1955). In 1957, Bromer and coworkers identified its amino acid sequence (Bromer et al., 1957).

Chemistry and Biosynthesis

Glucagon is a polypeptide hormone containing 29 amino acids. Its structure is highly preserved in different species, and the different glucagons differ usually only by one or two amino acids. Glucagon is synthesized in the alpha cells of the islets of Langerhans as a prohormone with a molecular mass of 18 kilodaltons. The exact mechanism of glucagon secretion into the blood is still controversial (Walker et al., 2011). Glucagon, which bears striking structural similarity to secretin, gastric inhibitory peptide, and vasoactive intestinal peptide, is coreleased with two major proglucagon fragments. Glucagon-like immunoreactive peptides are also synthesized in the gastrointestinal tract.

Secretion

Glucagon is a hyperglycemic hormone and as such is antagonistic to the action of insulin. The special arrangement of beta and alpha cells in the islet makes a close interaction of both hormones possible, and both glucagon and insulin exert a major regulatory function on metabolism (for review, see Walker et al., 2011). It seems that, for many metabolic effects, the ratio of insulin to glucagon is important for the regulation of cellular responses (Smith, 1989). In general, glucagon secretion is stimulated by low glucose concentrations, by protein intake, and by lowered fatty acid concentrations. Glucagon secretion is also under control of intestinal hormones and neurotransmitters (Unger and Orci, 1989).

Mechanism of Action

Glucagon is a major antagonist of insulin action. Glucagon stimulates glycogenolysis, gluconeogenesis, and lipolysis. Administration of glucagon leads to a rapid increase in intracellular cyclic adenosine monophosphate (cAMP) concentrations, which is followed by activation of cAMP-dependent protein kinase and subsequent substrate phosphorylation (Smith, 1989).

Metabolism

Glucagon is degraded in liver, kidney, plasma, and at its receptor on the plasma membrane. Its half-life in plasma is approximately 3–6 minutes (Unger and Orci, 1989).

Preparations and Dosage

Glucagon, in the past, was extracted from beef and pork pancreases, but now it exists in recombinant form. Glucagon for injection (Eli Lilly) contains glucagon as the hydrochloride and is dispensed in 1 mg vials. It is packaged with diluent containing glycerin, Water for Injection, and hydrochloric acid. One mg is usually administered to dogs for insulin-induced hypoglycemia.

Therapeutic Uses

Glucagon can be used for the treatment of insulin-induced hypoglycemia when dextrose is unavailable. It is only useful as a hyperglycemic agent in those patients that have sufficient hepatic glycogen. Glucagon should not be used in hypoglycemia due to insulinoma or in patients with pheochromocytoma, because it can induce hormone release from the tumors. In humans it is also used for radiographic and endoscopic examination of the gastrointestinal tract, because it acts as a smooth muscle relaxant. Glucagon has been used in small animals for double-contrast gastroscopy (Evans and Biery, 1983); however, a beneficial effect for endoscopic examinations in the dog could not be confirmed (Matz et al., 1991).

Adverse Effects

Glucagon is relatively free of adverse effects. Generalized allergic reactions have been reported in human patients after glucagon administration. Anecdotally, vomiting has been observed in some dogs and cats.

Glucagon-Like Peptide 1 and Dipeptidyl Peptidase 4 Inhibitors

Glucagon-like peptide 1 (GLP-1) is an incretin that augments insulin secretion after food intake. It also inhibits glucagon release and gastric emptying. Because of its poor stability, it cannot be used therapeutically. However, exendin-4 (Exenatide; Byetta®, Eli Lily/Amylin), a synthetic version of a peptide isolated from the saliva of the Gila monster, has been shown to bind and activate the known human GLP-1 receptor and is now used therapeutically for the treatment of type 2 diabetics. In people, it is injected subcutaneously twice daily within 60 minutes before the morning and evening meal. An exenatide extended-release formulation (Bydureon®**)** has been approved in 2012 for once-weekly treatment of type 2 diabetes. In an unblinded study, the treatment effect of exenatide added to glargine insulin in diabetic cats was not significantly greater than that seen in glargine placebo-treated cats (Riederer et al., 2016). Because the half-life of GLP-1 is very short, a new class of drugs has been developed, the dipeptidyl peptidase 4 (DPP-4) inhibitors. Their mechanism of action is thought to result from increased incretin levels (GLP-1 and glucose-dependent insulinotropic polypeptide, GIP)leading to increased insulin concentrations (Valk, 2007). Sitagliptine (Januvia®, Merck)was the first to be approved for use in human type 2 diabetics; now there are several more. It has been suggested that GLP-1 based therapies, including DPP-4 inhibitors, lead to pancreatitis and malignant transformation of cells, resulting in an increased risk for pancreatic and thyroid cancer (Elashoff et al., 2011). There are no reports of their use in diabetic pets.

Somatostatin

History

Somatostatin was isolated from ovine hypothalamus in 1973 by Brazeau and coworkers (Brazeau et al., 1973). Soon it became evident that somatostatin was not only present in hypothalamus but also in other tissues, including the pancreas (Hökfeldt et al., 1975; Arimura et al., 1975). It became apparent that the islet delta cells, first described by Bloom (1931), secreted this hormone, which inhibited insulin and glucagon secretion (Koerker et al., 1974).

Chemistry and Biosynthesis

Somatostatin is synthesized as a high-molecular weight precursor, which is differently processed in a tissue-specific manner. In the pancreas and hypothalamus it is cleaved mainly to a 14-residue peptide containing an internal disulfide bridge, whereas in the gastrointestinal tract the predominant form is a 28-residue peptide. Very little is yet known about the regulation of its biosynthesis.

Secretion

Much still needs to be learned about the regulation of somatostatin secretion. In the pancreas, somatostatin may act in a paracrine or endocrine manner when released from the delta cells (Hauge-Evans et al., 2009).

Mechanism of Action

The mechanism of action by which somatostatin inhibits insulin and glucagon release from the islet cells is not well understood. There is evidence that somatostatin inhibits insulin release by decreasing Ca influx through voltage-gated calcium channels, an action which is mediated through a G-protein (Hsu et al., 1991). It has been shown that somatostatin inhibits insulin gene expression through a posttranslational mechanism, however, at concentrations well above those measured in the peripheral blood. Somatostatin release is stimulated by most nutrients, including glucose, amino acids, and a fat–protein meal. Somatostatin also inhibits growth hormone release and a variety of gastrointestinal hormones (for a review see Long, 1987).

Metabolism

Somatostatin is metabolized in the liver (Ruggere and Patel, 1985). It has an apparent half-life of 3–5 minutes (Harris, 1994).

Preparations and Dosage

The only preparation available with pharmacological actions similar to somatostatin is the synthetic agent octreotide acetate (L-cysteinamide; Sandostatin® Injection, Sandoz Pharmaceuticals), a cyclic octapeptide prepared as a clear solution of octreotide acetate in buffered saline for deep subcutaneous injection. In humans, peak concentrations are reached within 0.4 hours; the half-life is approximately 1.5 hours in humans and approximately 75 minutes in dogs (for a review see Long, 1987). The duration of action of octreotide is variable but can extend up to 12 hours.

Therapeutic Uses

In humans, octreotide acetate is used most frequently in patients with VIP(vasoactive intestinal polypeptide)oma and carcinoid.

In dogs, octreotide has been used for the treatment of some insulinomas and for gastrinomas. Not all of the tumors treated responded to octreotide, likely because of the absence of specific receptors. Octreotide has not proven to be useful in the treatment of acromegaly. The dose for the treatment of insulinomas is 10–20 μg BID to TID; in gastrinomas 20–60 μg TID have been recommended (Lothrop, 1991). Octreotide has also been used in ferrets and cats with insulinomas with mixed results.

Adverse Effects

In humans, gastrointestinal problems are most frequently encountered after octreotide administration. Side effects in dogs have not been described.

Miscellaneous

Diazoxide (Proglycem®; Merck) is 7-chloro-3-methyl-2*H*-1,2,4-benzothiadiazine 1,1-diazoxide. It is a white powder practically insoluble in water. This nondiuretic benzothiadiazine inhibits insulin release from the beta cell by increasing the permeability to potassium, thereby hyperpolarizing the cell membrane (Trube et al., 1986). It also has extrapancreatic effects and promotes hepatic glycogenolysis and decreases glucose uptake in the liver (Altzuler et al., 1977). It is used in the treatment of insulin-secreting tumors. The initial dose of diazoxide is 10 mg/kg body weight divided into two daily doses. This dose can be increased gradually but should not exceed 40 mg/kg daily. Diazoxide is available as 50-mg capsules or as a suspension containing 50 mg/ml diazoxide.

Toceranib phosphate (Palladia®, Zoetis) (Z)-5-[(5-Fluoro-2-oxo-1,2-dihydro-3H-indol-3-ylidene)methyl]-2,4-dimethyl-N-(2-pyrrolidin-1-ylethyl)-1H-pyrrole-3-carboxamide phosphate is a tyrosine kinase inhibitor. It has been approved for the treatment of mast cell tumors in dogs but has been anecdotally also used to treat insulinomas. There are no scientific studies about the success of this drug in dogs with insulinomas.

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Section 9

Chemotherapy of Microbial Diseases

31

Antiseptics and Disinfectants

Megan E. Jacob

Introduction

Cleansers, antiseptics, and disinfectants play critical roles in preventing infectious disease transmission in veterinary medicine. From use as a presurgical scrub to disinfection after an outbreak, these products are relied upon by veterinarians for safe and effective germicidal activity. The beneficial effects of cleansing or disinfecting practices have been known for many years; the efficacy of hand washing was demonstrated as early as the 1840s by Ignaz Semmelweis, a Hungarian obstetrician. Following Pasteur's identification of infective agents as the cause of disease, Joseph Lister suggested the use of antiseptics in the field of surgery. His treatment of the hands with 1 : 20 carbolic lotion and his initiation of methods for chemical sterilization of bandages, dressings, and surgical instruments and for antisepsis of wounds began aseptic surgery.

Cleansers, antiseptics, and disinfectants are differentiated by their intended use and characteristic properties, not by their chemical content. A cleanser aids in physical removal of foreign material and is not necessarily a germicide. An antiseptic is a biocide applied to living tissue, whereas a disinfectant is a biocide applied to inanimate objects. Because certain antiseptics may be inactivated on inanimate surfaces and because certain disinfectants are hazardous to living tissue, the two should not be used interchangeably; however, these products may still have a very similar chemical content. Even products with the identical active chemical moiety may be formulated in such a way (e.g., exposure time, concentration) as to prevent their interchangeable use. Products formulated as disinfectants (and sanitizers or sterilants) to be used on inanimate surfaces, objects, or instruments are regulated by the Environmental Protection Agency (EPA). Antiseptics for use on living tissue must be registered with the Food and Drug Administration (FDA), along with some chemicals used on critical and semicritical devices.

Different cleaning, antiseptic, and disinfectant protocols exists for many different clinics, farms, procedures, and uses in veterinary medicine; no one compound is applicable, appropriate, or effective for every use.

Cleansers

Cleansers contain surfactants or detergents that remove dirt and contaminating organisms by solubilization and physical means. Cleansers are often a critical step to proper disinfection or antisepsis as removing gross contamination from an area prior to disinfection or antisepsis treatment maximizes their efficacy. Depending on the application and use, cleansing may be sufficient.

Cleansers can be classified into three types based on the presence and charge of the hydrophilic portion of the molecule: anionic, cationic, and nonionic. Soaps are anionic surfactants of the general structure $\text{R-COO}-\text{Na}^+$. Dissociation in water to $\text{R-COO}-$ liberates a molecule with both a hydrophilic and a hydrophobic portion, which can emulsify and solubilize hydrophobic dirt, fat, and protoplasmic membranes. Once solubilized, this contamination can be rinsed away with water. The ability to solubilize membranes renders soaps antibacterial against gram-positive and acid-fast bacteria. The anionic nature of soaps, however, causes them to be inactivated in the presence of certain positive ions such as free Ca^+ in hard water and in the presence of cationic detergents. The mixture of soaps and quaternary ammonium compounds forms a precipitate, which terminates the activity of both compounds. Inclusion of antiseptic compounds in soap preparations has given them a wider antibacterial spectrum.

The quaternary ammonium compounds (QACs) are examples of cationic surfactants with germicidal activity. These compounds have been widely used as disinfectants (see Section Examples of disinfectant use in Veterinary Medicine). Cationic surfactants combine readily with proteins, fats, and phosphates and are thus of limited value in the presence of serum, blood, and other tissue debris (Huber, 1988). In addition, use with materials such as gauze pads and cotton balls makes them less germicidal owing to absorption of the active ingredients.

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Antiseptics

An antiseptic is a chemical agent that reduces the microbial population on skin and other living tissues. Because in most cases its mechanism of action involves nonspecific disruption of cellular membranes or enzymes, caution must be taken not to harm host tissue. An ideal antiseptic would have a broad spectrum of activity, low toxicity, high penetrability, would maintain activity in the presence of pus and necrotic tissue, and would cause little skin irritation or interference with the normal healing process.

The use of antiseptics has been suggested in situations which require maximal reduction of bacterial contamination (Larson, 1987) such as when defense mechanisms are compromised after surgery, during catheterization or insertion of other invasive implants, and in immunocompromised states due to immune defects, cytotoxic drug therapy, extreme old or young age, or extensive skin damage (burns and wounds).

Disinfectants

Disinfection is a process that eliminates most, if not all, pathogenic organisms, excluding spore forms, from an inanimate object. Disinfection is sometimes incorrectly confused with sterilization, a process that completely eliminates all microbial forms by a physical or chemical means. True chemical sterilization necessitates the use of an EPA-registered agent capable of killing all infective organisms, including fungal and bacterial spores, usually within 10 hours. Sometimes, however, chemical sterilants can be considered disinfectants when shorter exposure periods are used. The treatment of objects that are too large to soak in disinfectant, such as cabinets, exam tables, chairs, lights, and cages, is considered surface disinfection. Immersion disinfection is the immersion of smaller objects in disinfectant for sufficient time to kill the majority of contaminating, pathogenic organisms.

The ideal characteristics of a disinfectant includes a broad spectrum, fast action, activity in the presence of organic material (including blood, sputum, and feces), compatibility with detergents, low toxicity, low cost, ease of use, and residual surface activity. They should not corrode instruments or metallic surfaces or disintegrate rubber, plastic, or other materials, and should be odorless and economical (Molinari et al., 1982).

The ability to kill different classes of microorganisms further categorizes disinfectants into high, intermediate and low levels. High-level disinfection destroys all microorganisms except high concentrations of bacterial spores. Intermediate-level disinfection inactivates acid-fast microorganisms, including *Mycobacterium tuberculosis*, most viruses and fungi, but not necessarily bacterial spores. Low-level disinfection kills most bacteria, some viruses, and some fungi, but not tubercle bacilli or bacterial spores. In addition, low-level disinfection usually occurs in less than 10 minutes.

A second classification system is intended to divide instruments and patient-care items into three categories based on risk of infection involved in their use (Spaulding, 1968). In this system, items are classified as: (i) critical – those that enter or penetrate skin or mucous membranes (e.g., needles, scalpels), usually at a sterile site; (ii) semicritical – those that touch intact mucous membranes (e.g., anesthesia equipment, endoscopes); and (iii) noncritical – those that do not touch mucous membranes but may contact intact skin (e.g., stethoscopes, cages, tables, food bowls). In general, items classified as critical should be sterilized, semicritical items require high-level disinfection, and noncritical items require low to intermediate-level disinfection.

Popular Antiseptic and Disinfecting Agents

Alcohol

Alcohols are one of the most popular antiseptic and disinfecting products, used every day in veterinary clinics and laboratories. Although many alcohols are germicidal, the two most commonly used as disinfecting agents are ethyl and isopropyl alcohol. These compounds are both lipid solvents and protein denaturants. They kill organisms by solubilizing the lipid cell membrane and by denaturing membrane cellular proteins. Alcohols are most effective when diluted with water to a final concentration of 70% ethyl or 50% isopropyl alcohol by weight. It is thought that at greater concentrations, initial dehydration of cellular proteins makes them resistant to the denaturing effect (Molinari and Runnel, 1991). Alcohols have excellent antibacterial activity against most vegetative gram-positive, gram-negative, and tubercle bacillus organisms but do not inactivate bacterial spores. They are active against many fungi and viruses, principally enveloped viruses due to alcohol's lipid-solubilizing action.

The alcohols are not recommended for high-level disinfection or chemical sterilization due to their inactivity against bacterial spores and reduced efficacy in the presence of protein or other bioburden. Blood proteins are denatured by alcohol and will adhere to instruments being disinfected. Fatal *Clostridium* spp. infections have occurred postoperatively that were the result of contaminated surgical instruments that had been disinfected with alcohol containing bacterial spores (Nye and Mallory, 1923). After repeated and prolonged use, alcohols can damage the shellac mounting of lensed instruments, can swell or harden rubber and certain plastic tubing (Rutala, 1990), and can be corrosive to metal surfaces. Alcohols are flammable; thus caution must be taken in their storage and when used prior to electrocautery or laser surgery. In deciding between ethyl and isopropyl alcohol, it is important to consider isopropyl's inactivity against hydrophilic viruses, its less corrosive nature, and the abuse potential for ethyl alcohol (grain alcohol).

Both isopropyl and ethyl alcohol are also commonly used, effective antiseptics, with only subtle differences in their action. Because their effectiveness is drastically reduced by organic matter such as feces, mucus, and blood, they are most effective on "clean" skin. They produce rapid reduction in bacterial counts (Lowbury et al., 1974), with contact times of 1–3 minutes, resulting in elimination of almost 80% of organisms. Rapid evaporation limits contact time; however, residual decreases in bacterial counts are seen to occur after the alcohol has evaporated from the skin. Although alcohols are among the safest antiseptics, toxic reactions have been reported in children. Alcohol can be drying to the skin and can cause local irritation. In efforts to minimize this drying effect, emollients such as glycerine have been added with good results (Larson et al., 1986).

Halogens

Iodine and chlorine both demonstrate antimicrobial activity and are used as antiseptics or disinfectants. Elemental iodine has germicidal activity against gram-positive and gram-negative bacteria, bacterial spores, fungi, and most viruses. It exerts these lethal effects by diffusing into the cell and interfering with metabolic reactions and by disrupting protein and nucleic acid structure and synthesis. Iodine has a characteristic odor and is corrosive to metals. It is insoluble in water and thus is prepared in alcohol (tincture) or with solubilizing surfactants ("tamed" iodines). Tincture of iodine, used as early as 1839 in the French Civil War, is most effectively formulated as a 1–2% iodine solution in 70% ethyl alcohol. In this form, most (approximately 90%) bacteria are killed within 3 minutes of application. The antibacterial activity of this combination is greater than that of the alcohol alone. Tincture of iodine, however, is irritating and allergenic, corrodes metals, and stains skin and clothing. It is also painful when applied to open wounds and is harmful to host tissue; therefore, it can delay healing and increase the chance of infection. For these reasons, this preparation has fallen out of favor as an antiseptic or disinfectant. Strong tinctures of iodine have been used as blistering agents in the equine industry.

Efforts to reduce the undesirable aspects of tinctures while retaining the powerful killing action of iodine have led to the introduction of tamed iodines known as iodophors. In this preparation, iodine is solubilized by surfactants, which allow it to remain in a dissociable form. Application of this product allows for slow continual release of free iodine to exert its germicidal effects. The iodophors have a similar spectrum of activity to aqueous solution; are less irritating, allergenic, corrosive, and staining; and have prolonged activity after application (4–6 hours). Common solubilizing carriers include polyvinylpyrrolidone (called PVP-iodine or povidone-iodine, PI) as well as other nonionic surfactants, making iodophors excellent cleansing agents as well as antiseptics and disinfectants. Iodophor solutions retain their activity in the presence of organic matter at pH <4 (Huber, 1988). The water-soluble carriers have been postulated to interact with epithelial surfaces to increase tissue permeability, thereby enhancing iodine's killing efficacy.

Proper dilution to 1% iodine is necessary for maximum killing effect and minimal toxicity. More-concentrated solutions are actually less efficacious, presumably due to stronger complexation preventing free iodine release. It takes approximately 2 minutes of contact time for release of free iodine (Lavelle et al., 1975). Literature reports indicate that iodophors are quickly bactericidal, virucidal, and mycobactericidal but may require prolonged contact times to kill certain fungi and bacterial spores. Iodophors formulated as antiseptics are not suitable as hard-surface disinfectants, due to insufficient concentrations of iodine.

Consideration must be taken of iodine's ability to be systemically absorbed through the skin and mucous membranes. The extent of absorption is related to the concentration used, frequency of application, and status of renal function (the principal excretory route) (Swaim and Lee, 1987). Complications of iodophor absorption include increased serum enzyme levels, renal failure, metabolic acidosis (Pretsch and Meakins, 1976), and increased serum free iodide. If renal function is normal, serum iodine concentrations quickly return to normal. Clinical hyperthyroidism and thyroid hyperplasia have been reported after treatment with PI (Scheider et al., 1976; Altemeier, 1976).

Chlorine-containing solutions were first introduced by Dakin in the early 1900s in the chemical form of sodium hypochlorite. They are effective bactericidal, fungicidal, virucidal, and protozoacidal agents. The chemical forms most commonly used today include the hypochlorites (sodium and calcium) and organic chlorides (chloramine-T). In either form, the germicidal activity is due to release of free chlorine and formation of hypochlorous acid (HOCl) from water. The mechanisms of action of these compounds include inhibition of cellular enzymatic reactions, protein denaturation, and inactivation of nucleic acids (Dychdala, 1983). Dissociation of HOCl to the less microbicidal hypochlorite ion (OCl^-) increases as pH increases, and thus the solution may be rendered ineffective above pH 8.0 (Weber, 1950). Mixing NaOCl with acid liberates toxic chlorine gas, and NaOCl decomposes when exposed to light.

Low concentrations of free chlorine are active against M. tuberculosis (50 ppm) and vegetative bacteria (<1 ppm) within seconds. Concentrations of 100 ppm destroy fungi in less than 1 hour, and many viruses are inactivated in 10 minutes at 200 ppm. Household bleach is 5.25% (52,500 ppm); thus dilutions of 1 : 100 to 1 : 250 should result in effective germicidal concentrations although more-concentrated solutions are often recommended (1 : 10 to 1 : 100).

The use of the hypochlorites as disinfectants are limited by several characteristics. Chlorine solutions are corrosive to metals and destroy many fabrics. Because chlorine solutions are unstable to light, they must be prepared fresh daily. Hypochlorites are inactivated by the presence of blood more so than are the organic chlorides (Bloomfield and Miller, 1989). They have a strong odor and are not suitable for enclosed spaces. In addition, hypochlorites may lead to irritation of mucous membranes and may form toxic bioproducts when interacting with other chemicals. Despite these shortcomings, chlorine solutions are commonly used as low-level disinfectants on dairy equipment, animal housing quarters, hospital floors, and other noncritical items. Of 12 disinfectant solutions evaluated for their ability to kill the dermatophyte *Microsporum canis*, those containing hypochlorite were most effective. Also found effective were benzalkonium chloride and glutaraldehyde-based products; phenolics and anionic detergents were considered inadequate (Rycroft and McLay, 1991). The hypochlorites are not recommended for routine use as antiseptics because they are very irritating to skin and other tissues and they delay healing. There is, however, research to suggest diluted household bleach can be applied to control superficial pyoderma in dogs.

Several compounds from a class called N-halamines (oxazolidinones or imidazolidinones) have been developed, which are water-soluble solids that have been shown to be bactericidal, fungicidal, virucidal, and protozoacidal in water disinfection at low total halogen concentrations (1–10 mg/l). They are noncorrosive and tasteless and odorless in water. They are extremely stable in water even in the presence of organic loads. Their potential use in poultry processing to control *Salmonella* has been evaluated (Smith et al., 1990).

Biguanides

Chlorhexidine (Chx) is popular synthetic cationic antiseptic compound (1-1’-hexamethylenebis[5-(p-chlorophenyl)biguanide]) with better activity against gram-positive than against gram-negative organisms. The compound lacks sporicidal activity. Chlorhexidine kills bacteria by disrupting the cell membrane and precipitating cell contents. It has also been suggested that membrane-bound adenosine triphosphatases, specifically inhibition of the F1 ATPase, may be a primary target for Chx (Gale et al., 1981). It is active against fungi, fairly active against *M. tuberculosis*, but poorly active against viruses. The antibacterial activity of Chx is not as rapid as that of the alcohols; however, as a 0.1% aqueous solution, significant killing action is evident after only 15 seconds. Additionally, Chx solutions have the longest residual activity, remaining chemically active for 5–6 hours and retaining their activity in the presence of blood and other organic material. Being cationic, it is inactivated by hard water, nonionic surfactants, inorganic anions, and soaps. Dilution with saline causes precipitation and its activity is pH dependent. It has extremely low toxicity even when used on intact skin of newborns (O’Neill et al., 1982). Chlorhexidine is available in a detergent base as a 4% solution or as a 2% liquid foam. Traditionally, it has widely been used as a presurgical antiseptic, wound flush, and teat dip. Formulations of chlorhexidine and alcohol have also been described and appear to improve efficacy. Its use as a disinfectant are not well described.

Polyhexamethylene biguanide (PHMB) is a polymeric biguanide with activity against gram-positive and gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus equi*. PHMB rapidly kills bacteria by disrupting the cytoplasmic membrane resulting in leakage and precipitation of cellular contents (Broxton et al., 1983). PHMB has been used to treat infections in the eye, mouth, and vagina and has been formulated in contact lens disinfectants and mouth rinses. It was shown to be nontoxic as a component of an ear flush for dogs (Mills et al., 2005) and when impregnated in a gauze wound dressing, reduced growth of underlying gram-positive and gram-negative bacteria in vitro (Lee et al., 2004).

Aldehydes

Two related aldehyde disinfectants are formaldehyde and glutaraldehyde (GLT). Formaldehyde has antimicrobial activity both as a gas and in liquid form. Formalin, the aqueous form, is 37% formaldehyde by weight. It inactivates microorganisms by alkylating the amino and sulfhydryl groups of proteins and ring nitrogen atoms of purine bases (Favero, 1983). Formaldehyde is an effective but slow bactericide, virucide, and fungicide, requiring 6–12 hours contact time. It is effective against *M. tuberculosis*, bacterial spores, and most animal viruses, including foot-and-mouth disease virus. Its action is not affected by organic matter and it is relatively noncorrosive to metals, paint, and fabric. Formaldehyde alone is considered a high-level disinfectant and in combination with alcohol can be used as a chemical sterilant for surgical instruments. However, due to irritating fumes and pungent odor at low concentrations (approximately 1 ppm), and because the National Institute for Occupational Safety and Health requires it to be handled as a potential carcinogen, thereby limiting worker exposure time, formaldehyde's use as a disinfectant has been limited.

Glutaraldehyde, a saturated dialdehyde, is similar to formaldehyde but without some of its shortcomings. It has better bactericidal, virucidal, and sporicidal activity than formaldehyde. Its biocidal activity is related to its ability to alkylate sulfhydryl, hydroxyl, carboxyl, and amino groups affecting RNA, DNA, and protein synthesis (Scott and Gorman, 1983). Acidic GLT solutions are not sporicidal; thus, they must be "activated" by alkalinizing agents to a pH between 7.5 and 8.5. Once activated, these solutions have a limited shelf life (14 days) due to polymerization of the GLT molecules (Rutala, 1990). Newer formulations (stabilized alkaline GLT, potentiated acid GLT, GLT-phenate) have increased shelf life (28–30 days) and excellent germicidal activity (Pepper, 1980). GLT has gained wide acceptance in high-level disinfection and chemical sterilization due to several favorable properties, including wide spectrum of activity. Low surface tension allows GLT to penetrate blood and exudate without coagulating proteins. It retains its biocidal activity in the presence of organic matter. It is noncorrosive to metal, rubber, and plastic and does not damage lensed instruments. GLT solutions must be used in well-ventilated areas, since air concentrations of 0.2 ppm are irritating to the eyes and nasal passages (CDC, 1987). Contact times of less than 2 minutes for vegetative bacteria, 10 minutes for fungi, and 3 hours for bacterial spores were necessary using a 2% aqueous alkaline GLT solution (Stonehill et al., 1963). Activity against the tubercle bacillus was found to be somewhat variable; at least 20 minutes at room temperature is needed to reliably kill these organisms with 2% GLT. When used as a high-level disinfectant, a minimum of 1% GLT should be used. GLT-phenate formulations should be used with caution since they were shown to be less effective than other aldehyde solutions in decreasing bacterial counts from some medical instruments (Ayliffe et al., 1986). GLT disinfectants were found to more effectively reduce duck hepatitis B virus infectivity when they contained additives such as alcohol, an ammonium chloride derivative, and a surfactant (Murray et al., 1991). The caustic nature of both formaldehyde and GLT makes them inappropriate as antiseptics, and in fact, protective gloves should be worn when using the aldehyde disinfectants.

Gluteraldehyde and QAC combinations have been formulated (e.g., Synergize^{TM}, Preserve International, Reno, NV) and largely marketed as a cleaner and disinfectant for use in animal (e.g., swine and poultry) production facilities.

Oxidizing Compounds

Conflicting reports concerning hydrogen peroxide's efficacy as a germicide make evaluating its utility in disinfection and antisepsis difficult. Although it has been reported to have bactericidal (Schaeffer et al., 1980), virucidal (Mentel and Schmidt, 1973), and fungicidal (Turner, 1983) activity, the activity of hydrogen peroxide is nonpenetrable and short lived. For this reason hydrogen peroxide antiseptic use is most valuable in the initial treatment of recently contaminated wounds. Because 3% hydrogen peroxide has been shown to be damaging to tissues, including fibroblasts (Lineweaver et al., 1982), it is not considered suitable for routine wound care. It is, however, considered a stable and effective disinfectant and is used in the disinfection of soft contact lenses. More recently, accelerated hydrogen peroxide products have been formulated to also contain a surfactant and stabilizer, which improve antimicrobial activity. These products are being implemented in many veterinary clinic settings for use as a disinfectant.

Other oxidizing agents include potassium peroxymonosulfate (PPMS), an oxidizing agent used in disinfection systems of pools and hot tubs. More recently, it has been formulated with potassium chloride and organic acids and salts (i.e., sulphamic acid, malic acid, sodium hexametaphosphate, and sodium dodecyl benzene sulphonate) resulting in a disinfectant effective against over 580 infectious agents including viruses, gram-positive and gram-negative bacteria, fungi (molds and yeasts), and mycoplasma (EPA Master Label). It is marketed as a powder because it is stable in solution for approximately 1 week. It is not inactivated by organic challenge and has been found to be user friendly to both humans and animals. It is widely used as a high-level disinfectant for surfaces in laboratories, dental care facilities, and hospitals; for decontaminating laundry; for air disinfection; and in food processing and transport. Use of peracetic acid, sodium perborate, benzyl peroxide, and potassium permanganate have also been reported in human and veterinary literature.

Phenols

Carbolic acid, a phenol, is the oldest example of an antiseptic compound. However, due to severe toxicity, it is no longer appropriate for use as an antiseptic. These agents act as cytoplasmic poisons by penetrating and disrupting microbial cell walls. Most commercially available phenolic products contain two or more compounds that act synergistically, resulting in a wider spectrum of activity, including against *M. tuberculosis*. Sodium o-phenylphenol is effective against staphylococci, pseudomonads, mycobacteria, fungi, and lipophilic viruses, and against ascarids, strongyles, and tichurids. Cresols are substituted phenols and are more bactericidal and less toxic and caustic than phenols. Phenolics are not recommended for disinfection of anything other than noncritical items, because of residual disinfectant on porous materials causing tissue irritation even when the items have been thoroughly rinsed, because of strong odors, and because of absorption into feed.

Triclosan (Irgasan DP 300; 2,4,4′ trichloro-2′-hydroxydiphenyl ether) is a chlorinated diphenyl ether or bisphenol that possesses high antibacterial activity particularly against many gram-positive (e.g., *Bacillus subtilis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli*, *Salmonella enterica* serotype Typhimurium, *Shigella flexneri*) as well as fungi and yeasts (Stewart et al., 1999). Triclosan has been used for over 30 years and was first introduced in the health care industry in a surgical scrub in 1972. However, recently there has been a rapid increase in the use of triclosan-containing products including soaps, disinfectants, deodorants, shampoos, and medical supplies. In addition, it can be incorporated into plastics (e.g., children's toys) and fibers to retard decomposition.

The mechanisms of action of triclosan have been debated, but it is likely that they are concentration dependent. At low concentrations, triclosan acts as a competitive inhibitor of bacterial enoyl-acyl carrier protein reductase, which is involved in the bacterial fatty acid elongation cycle. At higher concentrations, because of its lipophilicity, triclosan has been shown to incorporate into bacterial membranes to alter the physicochemical properties of the lipid bilayer, including perturbation of the packing and interaction between membrane phospholipids (Guillen et al., 2004). Resistance due to specific mutations (Heath et al., 1999) of the bacterial carrier protein has been demonstrated in *S. aureus* and *E. coli* (Fan et al., 2002). The clinical significance of this decreased sensitivity remains questionable since concentrations achieved during triclosan use are likely high enough for the generalized bactericidal activity to prevail. Decreased sensitivity has been demonstrated in bacteria that overexpress the AcrAB efflux pump (Wang et al., 2001). The possibility and data suggesting that these resistance mechanisms may not only confer resistance to triclosan but also to other antibiotics has led to concern about the ubiquitous use of this compound in detergents, toothpaste, and other household items.

Gases

Technologies using ethylene oxide or hydrogen peroxide gas plasma for sterilization have been described. These products may be toxic and are not appropriate for antisepsis but could be useful in the treatment of temperature-sensitive medical devices or equipment. Ethylene oxide (C_2H_4O) is a water-soluble flammable gas. Mixing ethylene oxide with carbon dioxide or fluorocarbons reduce its flammability. Ethylene oxide kills bacteria, fungi, yeasts, viruses, and spores. Bacterial spores are only two to ten times more resistant to the cidal activity than are vegetative cells. It has been shown that the relative humidity of the microenvironment is critical to microbial susceptibility to ethylene oxide. Activity is decreased in the presence of organic matter due to interaction with proteins and nucleic acids. Care must be used to contain the gas as it has an irritant effect on the skin and eyes and may cause headaches and nausea. Hydrogen peroxide gas plasma has also been reported to have efficacy against a broad range of microorganisms, including bacterial spores.

Formaldehyde gas inactivates viruses, fungi, bacteria, and bacterial spores. Its activity is dependent on relative humidity and its efficacy is thought to peak at less than 50% relative humidity. Formaldehyde has been used for disinfection of hospital linen and for terminal disinfection in certain food-producing industries (see Section *Salmonella*). Propriolactone, methyl bromide, and propylene oxide have also been used as gas disinfectants.

Factors Affecting Efficacy of Antiseptics and Disinfectants

As indicated above, many factors can influence the efficacy of antiseptics and disinfectants. Often, more than one of these factors is contributing to the efficacy of an antiseptic or disinfecting product in a clinical setting.

Concentration

In general, the time needed to kill an organism is inversely dependent upon antimicrobial concentration; however, for certain compounds a small decreases in concentration may result in large increases required for killing whereas other compounds are less sensitive to changes in concentration. Alcohols and phenolics are very concentration dependent, whereas QACs, aldehydes, and chlorhexidine are less sensitive.

Table 31.1 Q_{10} coefficients for selected disinfectant/antiseptic compounds

Compound	Q_{10} Coefficient
Formaldehyde	1.5
β-propriolactone	2–3
Ethylene oxide	2.7
Phenol and cresol	3–5
Aliphatic alcohols	30–40

Exposure Time

A minimum contact time is required for efficacy of most antiseptics and disinfectants. All registered EPA disinfectants are labeled with an appropriate contact time. Reducing the exposure time below recommended times could lead to incomplete germicide activity.

Temperature

Increased temperature results in increased antimicrobial activity. This relationship can be described by $Q_{(T2-T1)}$ = (time to kill at T_1) / (time to kill at T_2), where T_2 and T_1 are two different temperatures in centigrade degrees. This equation is commonly referred to as the Q_{10} coefficient and describes the change in activity caused by a 10°C rise in temperature. Table 31.1 lists the Q_{10} coefficient for certain disinfectant compounds.

pH

The pH at the site of action may affect a compound's activity by two mechanisms, influencing the compound or the microbial cell. Molecules such as phenols, and certain acids, including hypochlorous acid (bleach), are effective only in the unionized form, thus as pH increases they become less efficacious. Glutaraldehyde is more potent at alkaline pH but is more stable at acid pH. Increased pH results in higher numbers of negative charges on cell surfaces with which positively charged molecules, such as QACs and chlorhexidine, can interact, thereby increasing their activity. Lastly, in a process similar to absorption through any cell membrane, pH can effect partitioning from the bathing solution into the cell's interior.

Contamination

The most important step to maximize antiseptic or disinfectant efficacy is thorough cleaning and washing of the site or area prior to application. Organic matter such as blood, pus, feces, soil, food, and milk, are believed to directly reduce the activity of antimicrobial compounds via a chemical reaction which results in a smaller amount of compound available for killing microorganisms or by spatial nonreaction (the inability of the disinfectant molecule to get to the organism). Certain compounds (hypochlorites and iodines) are more susceptible to this type of interference than others. Glutaraldehyde is less affected by organic contamination than other compounds and is therefore useful for instruments whose surface or design make it impossible to thoroughly clean. In a veterinary setting, contamination can make disinfection of large animal facilities difficult and may require removal of surface layers of soil and bedding for complete treatment. The presence of inorganic ions, Ca^{+2}, Mg^{+2}, Na^{+}, and Cl^{-} may be physically incompatible with certain antiseptics/disinfectants and therefore dilution with either hard water or saline solutions may render these formulations ineffective.

Organism Type

The sensitivity of different organism types (bacteria, virus, fungi, and spores) have been previously discussed for levels and types of disinfecting agents. Within an organism type, differences between genera and species also exist, which may render a particular disinfection process ineffective against certain microbes yet effective against others. Gram-positive bacteria are in general less resistant to disinfectant and antiseptic compounds than are gram-negative organisms due to a less complex and less lipid-rich cell wall. Staphylococci are less susceptible to alcohols, glycols, and ethylene oxide than are other cocci. Of the gram-negative bacteria, *Pseudomonas aeruginosa* have been identified as more resistant to antimicrobial agents, especially QACs, than other species. Mycobacteria, due to the unusual and hydrophobic nature of their cell wall, are highly resistant to many compounds. Bacterial sporicides include the aldehydes, hydrogen peroxide, hypochlorites, iodine, acid alcohol, and ethylene oxide. By inhibiting germination or spore outgrowth, phenols, QACs, biguanides, and alcohols are sporostatic. The efficacy of most germicides against bacterial spores increases with temperature; however, the most effective method against bacterial spores is moist heat (115°C autoclaving). Fungi are sensitive to chlorine, phenols, iodine compounds, ethylene oxide, and the aldehydes, whereas QAC are fungistatic. Fungal spores are resistant to most disinfectants. The sensitivity of viruses to disinfectant compounds relates to the composition of the viral envelope. The lipid-enveloped viruses are readily inactivated by lipophilic agents such as ether, chloroform, phenols, QACs, and even detergents. The nonenveloped viruses are resistant to these agents but are sensitive to chlorine and the aldehydes. Formaldehyde and β-propriolactone are used to inactivate viruses in the production of viral vaccines utilized in veterinary medicine.

Formation of Biofilms

Bacteria present on metal or other surfaces may form a biofilm (Mafu et al., 1990) that is an adherent slimy layer consisting of an organic polymer matrix in which microbes are embedded. In addition, the intercellular matrix contains products of cellular metabolism including ions, nutrients, and enzymes such as polysaccharases, proteases, and β-lactamases. Differing cellular densities and extracellular concentrations of factors results in diverse cellular phenotypes with regard to growth rate, nutrient deprivation, etc. Bacteria in biofilm are less sensitive to disinfectant inactivation than are those grown in culture broth (i.e., planktonic). Proposed reasons for this increased resistance include decreased diffusion of disinfectant solution through polymer matrix preventing centrally located cells from being exposed to lethal concentrations of the compound. Chemical or enzymatic modification by extracellular components or decreased inherent microbe susceptibility due to slow growth rate or starvation responses may also contribute to increased bacterial survival in biofilms (Gilbert et al., 2002).

Resistance to Antiseptics and Disinfectants

Similar to resistance towards traditional antibiotics, bacteria can acquire genes that are associated with resistance toward antiseptic and disinfectant compounds. In general, acquisition of a genetic element (e.g., plasmid, transposon) or a chromosomal mutation results in bacteria with reduced susceptibility or increased tolerance; acquired resistance may not mean failure of an antiseptic or disinfectant, however. Concentrations of disinfectants in practice are generally much higher than the cidal concentration required to kill bacteria in vitro.

Staphylococcus aureus has been shown to be resistant to triclosan, quaternary ammonia compounds, and chlorhexidine (Heath and Rock, 2000; Suller and Russell, 1999, 2000). There are also reports of low-level resistance to QACs and chlorhexidine in *Pseudomonas* species (Mechin et al., 1999; Bamber and Neal, 1999; Tattawasart et al., 1999). However, this resistance has been considered unstable and not clinically significant (Russell, 2000). The possibility that the mechanisms of resistance developed against a disinfectant or antiseptic could confer resistance to an antibiotic, however, is considered quite possible and potentially clinically disastrous. It may also be possible that resistance genes associated with disinfecting or antiseptic agents are located on the same genetic element as genes encoding for antibiotic resistance.

The mechanisms of action of antibiotics are well known and in most cases, take advantage of a single specific target (e.g., inhibitors of peptidoglycan, protein, and nucleic acid synthesis, inhibitors of RNA polymerase, DNA gyrase) in their ability to kill or suppress the growth of bacteria. This is in contrast to mechanisms of action of disinfectants and antiseptics, which are less well understood and often involve more general and multiple cellular targets (Denyer and Stewart, 1998). These include interactions with the cell wall or the envelope, disruption of membrane integrity, interruption of the proton-motive force, and inhibition of membrane enzymes, or as alkylating, cross-linking, and intercalating agents. Similarly, the mechanisms of resistance to antibiotics have been better characterized than those to biocides. Changes in the drug's target (e.g., methylation of the ribosome, penicillin-binding protein alterations), impermeability to the drug (e.g., intrinsic gram-negative resistance, biofilm formation), enzymatic modification or destruction of the drug (e.g., β-lactamases), and increased efflux of the drug can confer resistance to antibiotics.

As mentioned, there is evidence of resistance to disinfectants developing or being measured in vitro; however, because of redundancy in the number of targets for activity and the ability to achieve very high concentrations of biocide chemicals at the site of contamination, in vivo or clinical resistance to these compounds is not thought to be prevalent. However, because several of the mechanisms that allow for this resistance are common to those that provide resistance to antibiotics (efflux, impermeability, modification of target sites), the possibility for decreased efficacy to antibiotics is real.

β-lactam resistance in association with resistance to quaternary ammonia compounds has been demonstrated in *S. aureus* (Akimitsu et al., 1999). Triclosan-resistant mycobacteria were also resistant to isoniazid (McMurry et al., 1999), and there are many reports of biocide–antibiotic cross resistance in gram-negative bacteria. For example, *E. coli* that are resistant to triclosan (McMurry et al., 1998) or pine oil (Moken et al., 1997) have been shown to display the multiple antibiotic resistance (mar) phenotype. It is generally believed that in *E. coli* the mar phenotype is attributable to increased efflux due to up-regulation of the efflux pump (e.g., AcrAB-ToIC) (Okusu et al., 1996), which can cause resistance to β-lactams, chloramphenicol, fluoroquinolones, and tetracyclines.

The discovery of ciprofloxacin-resistant *E. coli* on farms with no previously reported quinolone exposure suggests that a disinfectant caused antibiotic resistance (Randall et al., 2005). Based on laboratory investigations designed to induce antibiotic resistance by repeated exposure to three different disinfectants, these authors conclude that, although bacteria became less sensitive to fluoroquinolones, this mechanism could not produce clinically resistant strains from fully susceptible ones.

They further conclude that the risk of disinfectant exposure giving rise to multiple antibiotic resistant bacteria is outweighed by the value of sanitation provided by these compounds.

Examples of Antiseptic Use in Veterinary Medicine

Several resources, including the Centers for Disease Control and Prevention and World Health Organization, outline the role of antiseptics and disinfectants in human health care settings; many of the principals discussed in those references could be applied to veterinary hospitals. Discussions and research on antiseptic and disinfectant practices specific to veterinary medical settings are on-going and recommendations are expected to become more refined. Below are several specific examples of the role of antiseptics and disinfectants in veterinary medicine.

Presurgical Skin Cleansers

Skin cleansers are important in the presurgical antisepsis of both the surgeon and the patient. Historically, the recommendations for presurgical antisepsis of the surgeon include two alternatives. The first involves an initial water-and-soap cleansing followed by use of an alcohol-based rub for at least 5 minutes. The second and more traditional method consists of a 5-minute chlorhexidine or iodophor hand scrub. Alcohol hand rubs are effective in immediately eliminating pathological bacteria on the hand skin surface and have a prolonged period of action superior to traditional antiseptics. Chlorhexidine techniques have the advantage that the active agents have residual bactericidal activity under surgical gloves; however, they have been shown to have negative effects on the skin of health care workers, resulting in tissue disruption, elimination of beneficial deeper microflora, and predisposition to colonization with pathogenic bacteria. For this reason, the World Health Organization (WHO) now recommends alcohol-based handrubs as the gold standard (WHO, 2009). The presence of organic material and dirt can decrease the effectiveness of most antiseptics; thus removal of gross contamination should precede any antiseptic scrub. Additionally, an important reservoir of dirt and bacteria that needs to be specifically addressed is the subungual space (McHinley et al., 1988).

Preoperative preparation of the veterinary patient varies depending on the surgical environment, yet attempts to achieve the optimal antiseptic cleansing can aid in limiting postsurgical infections. Contrary to human surgery, hair removal from the operative site is almost always a necessity with animal patients. Clipping hair is superior to shaving since it causes less damage and less favorable conditions for bacterial colonization of the surgical skin site (Alexander et al., 1983). Removal of gross contamination and dirt should precede use of antiseptics for previously mentioned reasons. Gentle antiseptic scrubbing should begin at the incision site and move outward over the entire surgical area. Consideration of proper antiseptic contact times should be made. A final antiseptic spray is often applied and left to dry on the surgical site. Despite even the most careful presurgical preparation, up to 20% of skin-resident bacteria may be unaffected by skin antiseptic cleansing (Smeak and Olmstead, 1984). Characteristics of an ideal skin antiseptic include broad spectrum, rapid killing, persistent lethal effect, cleansing effect, lack of skin irritation, noninhibition of healing, and activity in the presence of organic material.

In one study, three antiseptic combinations were evaluated for surgical preparation of canine paws (Swaim et al., 1991): 7.5% PI scrub/10% PI solution, 2% Chx acetate scrub/2% Chx diacetate solution, and tincture of green soap/70% isopropyl alcohol combinations were each shown to effectively reduce bacterial colony counts. The first two combinations were also effective in residual killing when applied under a sterile bandage for 24 hours. However, no significant advantage of applying the antiseptics 24 hours prior to surgery was shown. This is in contrast to results in human patients, where antiseptic cleaning the night prior to surgery has resulted in fewer wound infections (Garibaldi et al., 1988). A similar technique involving prophylactic antiseptic cleansing and wrapping of a limb overnight has been shown to reduce contamination of equine orthopedic surgical sites (Stewart, 1984). For surgery of the foot, additional reduction in bacteria load was achieved by removal of the superficial layer of the hoof; however, counts remained above a level that might predispose the surgical site to infection (Hennig et al., 2001). Antibacterial agents found in shampoos were shown to prevent infections caused by *S. intermedius* in a skin infection model in beagles. Shampoo containing 3% benzoyl peroxide was most effective, followed by shampoos containing 0.5% Chx acetate and iodine (1.0% polyalkyleneglycol-iodine) (Kwochka and Kowalski, 1991).

Another study compared the presurgical efficacy of chlorhexidine to a stabilized glutaraldehyde compound. Glutaraldehyde is most commonly associated with disinfection of inanimate objects; however, in its stabilized form it was noncorrosive, nonvolatile, nontoxic, biodegradable, stable, and highly microbiocidal at neutral pH. Stabilized glutaraldehyde, with and without alcohol, and chlorhexidine with alcohol had similar and significant ability to reduce and maintain surface bacteria levels and therefore were recommended for presurgical antiseptic prophylaxis in elective (noncontaminated) procedures (Lambrechts et al., 2004).

Treatment of Open Wounds

The treatment of open wounds is an important procedure in veterinary medicine. The processes involved in wound healing and proper wound care have been reviewed (Swaim and Wihalf, 1985; Berk et al., 1992). Issues involved in the decision of how to properly treat a wound include patient age and general health status, and the age, cause, size, and extent of contamination of the wound. Treatment options include surgical closure, bandaging (of different types), and irrigation or application of a varied group of topical agents, including saline, antiseptics, antibiotics, and local anesthetics. It is important to recognize that each wound has different characteristics and thus treatment must be individualized. For all wounds, however, a basic principle to which all caregivers should adhere is "above all, do no harm"; that is, any agent chosen should not impede the healing process. When treating a wound topically, a general guideline would be not to apply anything that should not be placed in the patient's conjunctival sac (Peacock, 1984).

The literature is divided concerning the utility of antiseptics in routine wound care. Some authors contend that this practice reduces the incidence of infections as a complication (Zukin and Simon, 1987), while others believe that any benefit is outweighed by the potential for these agents to cause tissue damage (Oberg and Lindsey, 1987). Once the healing process has begun, however, the use of more-benign agents may be indicated. Saline has been shown to be an effective means of eliminating debris and lowering bacterial counts (Stevenson et al., 1976). Hypertonic saline has also been proposed as a wound dressing (Lowthian and Oke, 1993). Archer et al. (1990) report that surface colonization of wounds does not impede healing and thus recommend a move away from potentially damaging antiseptics.

Many reports in the literature discuss potential toxic and harmful effects of antiseptics on fragile healing tissues, making their use controversial. A 5% PI solution inhibited local leukocyte migration, fibroblast activity, and wound cellularity (Viljanto, 1980). In vitro, neutrophil migration was inhibited at concentrations greater than 0.05% (Tvedten and Till, 1985), whereas 1% PI killed fibroblasts and resulted in weaker wound breaking strength (Lineweaver et al., 1985). Detergent scrubs containing PI and other surfactants were found to damage wound tissue and therefore are not recommended for wound care (Rodheaver et al., 1982). A maximum of 1% PI solution has been recommended as the most effective and least tissue-toxic dilution for wound irrigation (Swaim and Lee, 1987). Because antibacterial activity lasts 4–6 hours, repeated treatment is necessary for optimal results.

Chlorhexidine's residual activity (possibly by binding to proteins of the stratum corneum) and its activity against many organisms, make it a useful wound treatment. In an experimental wound infection model, wounds irrigated with 0.05–1% Chx diacetate solution had fewer infections than those treated with 0.1–0.5% PI. Concentrations of Chx gluconate 0.5% or greater were effective against *S. aureus* in vitro; however, concentrations above 0.05% were lethal to equine fibroblasts (Redding and Booth, 1991) and in a wound model in pigs. Unfortunately, it also delayed healing to a greater extent than other solutions tested, including PI (Archer et al., 1990).

Chlorine solution, such as sodium hypochlorite, was used as an effective wound flush in World War I. Full strength Dakin's solution (0.5% NaOCl) kills bacteria and fibroblasts, as well as retarding epithelialization in vivo in rats (Lineweaver et al., 1985). Other studies have shown low concentrations (0.025–0.0025%) to be toxic to neutrophils, fibroblasts, and endothelial cells, prompting one author to recommend abandoning the use of NaOCl as an irrigant (Kozol et al., 1988). In contrast, a concentration of 0.025% NaOCl was shown to be bactericidal while having no in vitro or in vivo tissue toxicity, suggesting a modified Dakin's solution may be a safe and effective fluid dressing (Heggers et al., 1991). Chloramine-T (Chlorazene) was shown to reduce in vitro *Pseudomonas aeruginosa* growth and the ability of the bacteria to colonize experimentally created wounds in guinea pigs. Additionally, Chlorazene was seen not to delay the healing of these wounds at a concentration of 0.03% (Henderson et al., 1989). Thus, it was concluded that this preparation should have no effect on healing of wounds when used to sanitize hydrotherapy units. More recently, sodium hypochlorite solutions have been proposed and evaluated for treatment of atopic dermatitis and recurrent pyoderma in dogs, as it is highly effective against *Staphylococcus* species in vitro. The use of this and other topical antiseptic products is being increasingly investigated as veterinarians continue to struggle with antibiotic resistant (including methicillin-resistant *Staphylococcus* spp.) organisms associated with skin infections.

Examples of Disinfectant Use in Veterinary Medicine

Disinfectants are widely used in veterinary medicine on floors, tables, walls, surgical equipment and other instruments before storage, and for disinfection of animal housing facilities. For effective germicidal activity, manufacturer recommendations regarding contact time, dilution, and useful life of a disinfectant solution should be followed. The best disinfectant for a particular situation will depend on the surface's shape, structure, chemical reactivity, and use, as well as on the type of contaminating organisms anticipated. In addition, in almost all instances, disinfection is only effective after the removal and cleaning of organic debris.

Although detailed descriptions of guidelines for disinfectant use in all circumstances is beyond the scope of this chapter, it is justified to provide several examples of their use in situations involving microbes that cause significant health concerns or that can be easily transmitted.

Salmonella

Salmonella species are well known by veterinarians and controlling transmission of the organism is important in a variety of settings. There are many *Salmonella* serotypes, some of which may be associated with an increased concern for transmission. An important food-borne pathogen, control of *Salmonella* on production animal facilities, even when animals are asymptomatically shedding the organism in feces, is important for public health and consumer confidence. Zoonotic transmission in a veterinary clinic setting from a cat to technical staff has also been reported (Cherry et al., 2004). In other situations, patients are symptomatic with a *Salmonella* infection and efforts to prevent transmission to other patients, particularly in hospital or boarding facilities, is critical. Several highly publicized *Salmonella* outbreaks have caused interruptions in services at veterinary teaching hospitals, prompting the search for effective methods of detection, prevention, and disinfection. Environmental contamination (Patterson et al., 2005) or affected individuals (Schott et al., 2001) may have been the initiating events; however, housing of sick and immunocompromised patients as well as incomplete disinfection likely contributed to routine shedding developing into epidemics. Sites and methods of sampling for monitoring for *Salmonella* contamination have been proposed and a high frequency of positive results have been recorded (~50%). However, this high percentage may reflect the sampling and detection method and not necessarily disease risk. The ability to decontaminate a veterinary hospital quickly, efficiently, and effectively is paramount to preventing loss of income and public confidence and to ensuring high quality treatment of the veterinary population. Following one outbreak (in 1996 at Colorado State Veterinary Teaching Hospital) the facility was at least partially closed for 3 months to allow manual decontamination/disinfection.

Disinfectant footbaths have been used as a hygiene barrier to prevent spread of microbes in veterinary hospital environments. Footbath efficacy has been shown to be dependent upon the disinfectant used and the compliance with which it is utilized. In one study, a peroxygen compound was shown to be more effective than a QAC; however, a maximal reduction in contamination of only 75% was observed (Morley et al., 2005). These results suggest that footwear hygiene can be improved through appropriate use of disinfectant footbaths, but it should not be relied on as the only method of controlling the spread of infectious agents. Mist application of a 4% peroxymonosulfate compound was shown to be an effective method of eliminating artificially induced contamination of an animal holding facility (Patterson et al., 2005).

Glutaraldehyde was found to be the most effective compound in reducing *Salmonella enteritidis* and *S. senftenberg* bacterial load in a study designed to mimic worst-case conditions in disinfecting poultry houses (Gradel et al., 2004). Four types of materials (e.g., concrete, wood) were contaminated with bacteria mixed with several types of organic matter (e.g., feed, egg yolk) and disinfection was attempted at high and low temperatures. Formaldehyde was considered effective even at low temperatures despite reports that a minimum temperature of 16°C is required for activity, whereas a peroxygen compound was found to be least effective except for one material/organic matter combination. This lack of efficacy was attributed to peroxygen compounds inactivation in the presence of organic matter.

In a similar study that investigated disinfection of poultry transport containers, real-world conditions were created by testing five compounds against bacteria growing isolated and in a biofilm. Although halogen compounds and QAC were effective against artificially contaminated surfaces, after the biofilm had matured, only sodium hypochlorite or an iodine-containing disinfectant was able to achieve 100% reduction. In the ultimate test of disinfectant activity in the face of organic matter, sodium carbonate, ammonia, and sodium hydroxide were shown to reduce food-borne pathogen load in cattle manure (Park and Diez-Gonzalez, 2003).

Avian Influenza

The spread of avian influenza (including highly pathogenic avian influenza, HPAI) among poultry populations and is very concerning and problematic. As an enveloped virus, the orthomyxoviridae, including influenza viruses, are very sensitive to most detergents and disinfectants. They are readily inactivated by pH, heating, and drying. The US Environmental Protection Agency currently reports approximately 200 products registered for disinfection use against avian influenza. These products are typically intended to be used by poultry producers to disinfect their facilities after an outbreak. Classes of disinfectants considered effective at destroying avian influenza virus include alcohols, phenolics, oxidizing agents, and dilute acids. However, flu viruses are well protected from inactivation by organic material, and infectious virus can be recovered from manure for up to 105 days. It is therefore suggested that complete removal of all organic material is part of any effective disinfection procedure. Contaminated litter and manure should be composted or buried to ensure that it does not spread infectious virus.

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32

Sulfonamides and Potentiated Sulfonamides

Mark G. Papich

The sulfonamides are one of the oldest groups of antimicrobial compounds still in use today. Sulfanilamide, an amide of sulfanilic acid, was the first sulfonamide used clinically. It was derived from the azo dye Prontosil. Other sulfonamides also share the same structure and the "sulfonamide" structure is prevalent among other drug classes, including nonsteroidal antiinflammatory drugs (NSAIDs), anticonvulsants, and diuretics. Sulfonamide antimicrobials have been in clinical use for 50 years, but resistance is common when these drugs are used alone (without addition of trimethoprim or ormetoprim). Previous editions of this textbook should be consulted for a review of this extensive historical database. Clinical use of sulfonamides in dogs, cats, horses, and some exotic and zoo animals usually relies on the addition of trimethoprim (trimethoprim–sulfonamide) or ormetoprim (e.g., ormetoprim–sulfadimethoxine) to broaden the spectrum and increase antibacterial activity against bacteria that are resistant to either drug used alone. Technically, trimethoprim and ormetoprim are chemically called *diaminopyrimidines,* but they will be referred to by their respective names in this chapter. In companion animals, trimethoprim–sulfonamide combinations have all but replaced single or combination sulfonamide (triple-sulfas) treatment regimens. Sulfonamide administration is restricted in food animals, particularly dairy cattle, because of a concern for drug residues.

Pharmacology of Sulfonamides

All sulfonamides are derivatives of sulfanilamide (structurally similar to para-aminobenzoic acid), which was, in the 1940s, the first sulfonamide discovered to have antimicrobial activity. Note that in some countries and certain formularies outside the United States, different spellings have been used for sulfonamides (e.g., *sulphamethoxazole* for sulfamethoxazole; *sulphadiazine* for sulfadiazine; *sulphadimethoxine* for sulfadimethoxine, and so forth). This textbook uses the United States Adopted Names (USAN) and United States Pharmacopeia (USP) official names throughout.

Many structural derivatives of sulfanilamide with differing pharmacokinetic and antimicrobial spectrums have been used in veterinary medicine to treat microbial infections of the respiratory, urinary, gastrointestinal, and central nervous systems (Figure 32.1). Susceptible organisms include many bacteria, coccidia, chlamydia, and protozoal organisms, including *Toxoplasma* spp. Treatment of protozoa infections is discussed in more detail in Chapter 42 of this book.

Sulfonamides are white crystalline powders that are weak organic acids, with solubility in water that varies among the specific drugs (ranging from slightly soluble to practically insoluble), and have a wide range of pK_a values, as shown in Table 32.1. The pK_a values of these compounds and their ionization are important because – among other properties – the antibacterial activity, solubility, and protein binding have been associated with the pK_a value (Mengelers et al., 1997). Drugs with high pK_a are less soluble and exhibit lower protein binding; drugs with low pK_a tend to have higher protein binding. The sulfonamides all share a similar structure, which contains a $–SO_2$ group linked to a benzene ring, and a para NH_2- group on N-4. An attached pyrimidine ring may contain zero, one, or two methyl groups (sulfamethazine, sulfamerazine, and sulfadiazine, respectively), which may undergo hydroxylation during metabolism. The other major site of metabolism is acetylation of the para-NH_2, which can vary among species (for example, dogs do not acetylate, which is discussed in Section Metabolism). Acetylated forms of the drug tend to be less soluble.

The sulfonamides exhibit large variation in the extent to which they bind to plasma proteins. In general, the plasma protein binding is higher than other antimicrobials (>70% in many animals), and ranges from 90% (sulfadimethoxine in some species) to as low as 50% (sulfamethazine in some species). In horses, the protein binding of trimethoprim was 20–30% and for sulfadiazine was 18–30% (Winther et al., 2011). Because they

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Companion Website: www.wiley.com/go/riviere/pharmacology

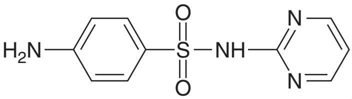
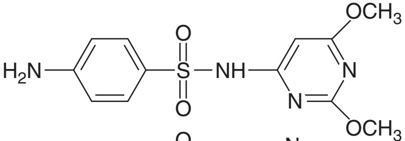
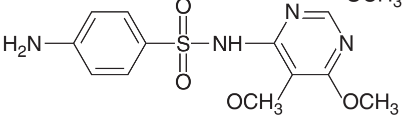
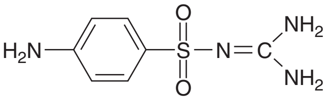
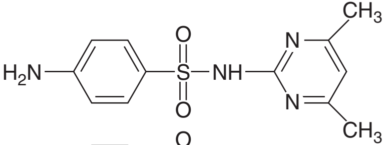
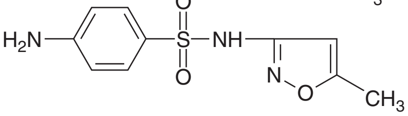
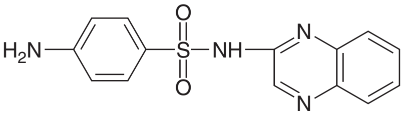
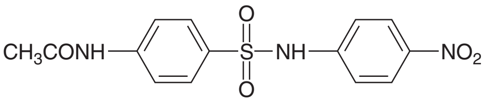
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Sulfadiazine	4-amino-*N*-2-pyrimidinylbenzenesulfonamide ($C_{10}H_{10}N_4O_2S$) [250.28]	
Sulfadimethoxine	4-amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_4S$) [310.33]	
Sulfadoxine	4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_4S$) [310.34]	
Sulfaguanidine	4-amino-*N*-(aminoiminomethyl)-benzenesulfonamide ($C_7H_{10}N_4O_2S$) [214.24]	
Sulfamethazine	4-amino-*N*-(4,6-dimethoxy-2-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [278.32]	
Sulfamethoxazole	4-amino-*N*-(5-methyl-3-isoxazolyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [253.31]	
Sulfaquinoxaline	4-amino-*N*-2-quinoxalinyl-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [300.33]	
Sulfanitran	4′-[(*p*-nitrophenyl)sulfamoyl]acetanilide ($C_{12}H_{14}N_4O_2S$) [300.33]	

Figure 32.1 Sulfonamides and their structures.

Table 32.1 Physical chemistry properties of sulfonamides, trimethoprim, and ormetoprim

Drug	pK_a	Log P
Sulfanilamide	10.1	−0.072
Sulfadimidine	7.7	0.691
Sulfamerazine	7.0	0.812
Sulfadiazine	6.4, 6.5, 6.6	0.631
Sulfadimethoxine	6.3, 6.2	1.648
Sulfachlorpyridazine	6.1, 6.0	1.305
Sulfamethoxazole	5.7, 5.9, 6.0	1.396
Sulfisoxazole	5.0, 4.9	2.259
Sulfadoxine	6.1, 6.3	1.271
Sulfaquinoxaline	5.5	1.68
Trimethoprim	7.12, 7.6	0.91
Ormetoprim	na	1.23

The pK_a is the dissociation rate constant. For some drugs, more than one pK_a value is listed because of variation among sources. For pK_a values, all sulfonamides are weak acids; trimethoprim and ormetoprim are weak bases. Log P is the logarithm of the partition coefficient between an organic solvent (oil) and water. The higher the Log P, the more lipophilic is the drug. Some values are from Mengelers et al. (1997) and van Duijkeren et al. (1994a).

are weak acids, sulfonamides are more soluble in alkaline than in neutral or acidic pHs; water solubility is enhanced when the sulfonamides are formulated as sodium salts or when in solution in more alkaline environments. Some sulfonamide solutions have pHs between 9 and 10, prohibiting extravascular use. Because solubility is decreased in acidic pH, they may become particularly insoluble and crystallize in renal tubules when urine pH is low, especially when high doses are administered, or animals are dehydrated or acidemic. To minimize crystalluria, yet allow administration of high doses, they have been formulated in combination with other sulfonamides. Each sulfonamide in a mixture of sulfonamides exhibits its own solubility in solution (law of independent solubility); that is, sulfonamides do not significantly affect the solubility of each other, but the antimicrobial effect is additive; thus the use of “triple-sulfas” (three sulfonamides formulated in solution together) allows increased efficacy without a significant increased risk of adverse effects (Bevill, 1988).

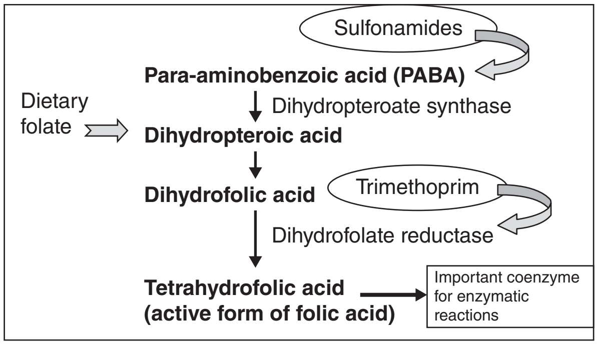


Figure 32.2 Simplified pathway for the action of trimethoprim–sulfonamide combinations. Sulfonamides provide a false substrate for para-aminobenzoic acid (PABA) inhibiting the synthesis to dihydropteroic acid, a precursor for synthesis to dihydro- and tetrahydrofolic acid. Trimethoprim inhibits the enzyme dihydrofolate reductase, an enzyme critical to the synthesis of tetrahydrofolic acid.

Mechanism of Action

Sulfonamides rely on the requirement of susceptible organisms to synthesize folic acid as a precursor of other important molecular molecules in the cell. Sulfonamides act as false substrates in the synthesis of folic acid. Trimethoprim and ormetoprim (diaminopyrimidines, discussed in Section Potentiated Sulfonamides) produce a synergistic effect when used together by inhibiting the enzyme dihydrofolate reductase.

Folic acid metabolism is presented in Figure 32.2. Para-aminobenzoic acid (PABA), pteridines, glutamic acid, and the enzyme dihydropterate synthase interact to form dihydropteroic acid, the immediate precursor to dihydrofolic acid. Dihydropteroic acid is enzymatically converted to dihydrofolic acid by dihydrofolate synthase, followed by another enzymatic conversion of dihydrofolic acid to tetrahydrofolic acid (THFA) via dihydrofolate reductase (DHFR). The combination of sulfonamides and trimethoprim inhibits formation of tetrahydrofolic acid at two steps. This action is synergistic and increases activity against organisms that could otherwise be resistant. Tetrahydrofolate is a coenzyme in a number of complex enzymatic reactions and also is a coenzyme in the synthesis of thymidylic acid (a nucleotide), which is a building block of DNA. Trimethoprim and sulfonamides are bacteriostatic by themselves; together, they can be bactericidal. Bacteria are more susceptible to this combination than to either drug when tested alone (White et al., 1981).

Trimethoprim–sulfonamides are formulated in a ratio of 1:5 (trimethoprim:sulfonamide). In the animal, it is usually cited that the optimum ratio to produce antibacterial activity is 1:20 (Bushby, 1980; van Duijkeren et al., 1994b). Testing for susceptibility using approved CLSI methods (CLSI, 2015) uses a ratio of 1:20 trimethoprim:sulfonamide. However, this ratio is often much lower in animals because the trimethoprim component is excreted faster than the sulfonamide and the optimum ratio may actually be much wider than the value of 1:20 cited in human medical references, and may be as low as 1:40.

Sulfonamide action is dependent on the chemical similarity with PABA. Therefore, sulfonamides act as a false substrate in this reaction and synthesis of THFA is inhibited. The sulfonamides are relatively safe to mammalian cells because mammals utilize dietary folate for the synthesis of dihydrofolic acid, and they do not require PABA. The enzyme dihydrofolate reductase of bacteria has a much higher affinity (50,000 to 60,000-fold, and in some references as high as 100,000-fold) for trimethoprim than mammalian dihydrofolate reductase.

The mechanism of action of sulfonamides on bacteria does not entirely explain the activity against protozoa. Sulfonamides may inhibit protozoal dihydrofolate synthetase. Protozoal dihydrofolate reductase also is susceptible to the action of trimethoprim, which may explain some of the effect to support the use of these drugs for protozoal infections (treatment of protozoa infections is discussed in Chapter 42).

Clinical Uses and Microbial Susceptibility

The spectrum of activity for the sulfonamides is broad, affecting gram-positive, gram-negative, and many protozoal organisms. Sulfonamides have been used clinically for approximately 50 years and many organisms once susceptible to the sulfonamides are now resistant. To increase the activity, most of the sulfonamides used in clinical practice are combinations with either trimethoprim or ormetoprim (diaminopyrimidines). These combinations (referred to in this chapter as *trimethoprim–sulfonamides,* but also referred to in clinical practice as *trimethoprim–sulfa* or simply abbreviated as *TMP/SU*) have increased the activity.

Administration of a single sulfonamide, or combination of sulfonamides, continues to be used in some livestock practices. In the United States, there are no approved formulations of trimethoprim–sulfonamides available for food animals, but trimethoprim–sulfadoxine is available in some countries.

The susceptibility/resistance patterns of sulfonamides and the trimethoprim–sulfamethoxazole combination against the most commonly encountered veterinary pathogens has been reported (van Duijkeren et al., 1994a, 1995; Bade et al., 2009; Winther et al., 2011). The activity of these agents has allowed for treatment of common respiratory infections, urinary tract and soft tissue infections, and intestinal infections (intestinal protozoa). Susceptible organisms include *Arcanobacterium*, *Bacillus* spp., *E. rhusiopathiae, L. monocytogenes, Streptococcus* spp., (*Streptococcus equi* subsp. *zooepidemicus* from horses), and protozoa (coccidia and *Pneumocystis carinii*).

The wild-type strains of following organisms are usually susceptible to the trimethoprim–sulfonamide (or ormetoprim–sulfonamide) combination: *Pasteurella* spp., *Proteus* spp., *Salmonella* spp., *Histophilus* (formerly *Hemophilus*), the protozoa *Toxoplasma,* and coccidia. Other bacteria that may be susceptible, but for which resistance can develop, include *Staphylococcus* spp., *Corynebacterium, Nocardia asteroides, Stenotrophomonas maltophilia,* and bacteria of the Enterobacteriaceae (*Klebsiella, Proteus, Enterobacter,* and *Escherichia coli*).

The organisms that are consistently resistant to trimethoprim–sulfonamide combinations include: *Pseudomonas* spp., *Chlamydia spp.,* and *Bacteriodes*. One should cautiously interpret trimethoprim–sulfonamide susceptibility for *Enterococcus spp*. Although *Enterococcus* may appear susceptible to trimethoprim–sulfonamides using in vitro tests, it escapes the antifolate activity of the drug in vivo by its unique ability to incorporate preformed exogenous folates (Wisell et al., 2008). Sulfonamides alone are not active against *Enterococcus* spp. Clinical failures are reported despite in vitro susceptibility and microbiology laboratories should not report the susceptibilities of *Enterococcus* to trimethoprim–sulfonamides.

The activity of trimethoprim–sulfonamides against anaerobic bacteria can be variable. When measured in vitro, trimethoprim–sulfonamides have good activity against anaerobic bacteria (Indiveri and Hirsh, 1986), but clinical results are not as good (Dow, 1988) because thymidine and PABA (inhibitors of trimethoprim–sulfonamide activity) may be present in anaerobic infections.

Trimethoprim–sulfonamides have been used to treat infections caused by protozoa (including *Toxoplasma gondii*) and intestinal coccidia. Trimethoprim–sulfonamide combinations have also been used to treat equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona.* (Use of pyrimethamine for treating EPM and treatment of protozoa infections is discussed in Chapter 42.)

Interactions Affecting Antimicrobial Activity

Components found in some tissue environments may inhibit trimethoprim–sulfonamide activity. For example, thymidine and PABA present in infected tissue – may interfere with activity. This has been demonstrated in tissue cages in horses. Ensink et al. (2005) showed an inability to eliminate the infection in an infected environment, despite in vitro sensitivity. They cited inhibitors – such as PABA and thymidine – present in abscessed and infected tissues that may inhibit the effects of these drugs. In another study in which trimethoprim–sulfadoxine was administered to cattle with infected tissue cages (Greko et al., 2002), it was shown that high levels of thymidine in the tissue cage fluid inhibited trimethoprim and compromised the ability to eradicate the infection.

Susceptibility Testing

For susceptibility testing, trimethoprim–sulfamethoxazole (1:20 ratio of trimethoprim:sulfamethoxazole) should be used, even when trimethoprim–sulfadiazine is used for therapy (CLSI, 2013, 2015). There are no quality control (QC) ranges developed for trimethoprim–sulfadiazine, and tests using trimethoprim–sulfamethoxazole are expected to give equivalent results. Winther et al. (2011) showed that there were no significant differences observed between the minimal inhibitory concentration (MIC) of sulfadiazine and sulfamethoxazole for individual bacterial strains, confirming that sulfamethoxazole is an effective surrogate for susceptibility testing of sulfadiazine. The CLSI susceptibility testing standards state that Mueller–Hinton agar containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and of trimethoprim, which may result in false-resistant reports (CLSI, 2013). Susceptibility testing agar that is as thymidine free as possible should be used. The current CLSI interpretive categories (CLSI, 2015) do not provide veterinary-specific interpretations; therefore, the human breakpoint is used by laboratories to predict susceptibility. For *Staphylococcus* spp. and the Enterobacteriaceae the susceptible breakpoint is ≤2/38 (trimethoprim/sulfonamide) and for *Streptococcus* spp. the breakpoint is ≤0.5/9.5 (trimethoprim/sulfonamide).

Drug Resistance

Resistance by many bacterial and protozoal organisms has become widespread due to the extensive use of sulfonamides over many years (Huovinen, 2001). Resistance occurs via efflux pumps, failure to penetrate the organism, and changes in target enzymes. Resistance can be transferable. Chromosomal resistance tends to occur slowly and confers resistance via impaired drug penetration into the microbial cell, producing an insensitive dihydropteroate enzyme and an increased production of PABA. Plasmid-mediated resistance, the most commonly encountered form of sulfonamide resistance, occurs quickly and manifests itself via the impaired drug penetration mechanism in addition to producing sulfonamide-resistant dihydropteroate synthase enzymes. If an organism becomes resistant to one sulfonamide, it is generally resistant to all other sulfonamides. Resistance to trimethoprim occurs via overproduction of the dihydrofolate reductase enzyme or synthesis of an enzyme that resists binding of the drug.

Pharmacokinetics of Sulfonamides

Pharmacokinetics of sulfonamides, trimethoprim, and related drugs used in veterinary medicine are listed in Tables 32.2, 32.3, 32.4, 32.5, and 32.6.

Oral Absorption

In dogs, absorption is excellent and not affected by feeding (Sigel et al., 1981). There has been considerable interest in the oral absorption of trimethoprim–sulfonamide combinations in horses and the effect of feeding. When trimethoprim-sulfonamides are administered to a horse that has not been fed, rapid absorption occurs, but is not as complete as for dogs or people. Nevertheless, oral administration is sufficient in horses to produce effective results. The fraction absorbed for trimethoprim was reported to be 67%, and for sulfadiazine 58%, but for both components the variability was high (van Duijkeren et al., 1994c). Oral absorption in another study in horses was 90.2% for intragastric administration and 74.45% for the oral paste (Winther et al., 2011). For trimethoprim in the same study it was 71.5% oral absorption for the intragastric administration and 46% for the oral paste (Winther et al., 2011). In that study the absorption of trimethoprim–sulfadiazine was likely diminished by feeding. When trimethoprim–sulfadiazine was administered to horses as an oral suspension and compared to the equine paste, the absorption from the suspension was higher for both drugs compared to the paste, that is 136% and 118% of the paste AUC concentrations for sulfadiazine and trimethoprim, respectively (McClure et al., 2015). In another study (van Duijkeren et al., 1994c) the oral paste was compared to two compounded formulations (mixed with syrup and water or carboxymethylcellulose gel). In this comparison, all three formulations were judged to be equivalent. When administered to horses that have been fed or when it is added to the horses' feed concentrate, a delayed and biphasic absorption is observed (van Duijkeren et al., 2002, 1995). When trimethoprim sulfachlorpyridazine was administered to horses, oral absorption was delayed, with the first peak appearing 1 hour after dosing and the second appearing 8–10 hours postdosing. Dual absorption peaks were not found after nasogastric administration (van Duijkernen et al., 1995). The best explanation for this phenomenon is that that there is an initial peak of absorption in the small intestine where much of drug absorption is known to occur. However, the drug that is bound to feed (adsorption) is unavailable for absorption until it travels to the cecum and, after digestion of the carbohydrates, the drug is released, producing a delayed and biphasic peak in absorption. Trimethoprim–sulfachlorpyridazine can bind to equine cecal contents 60–90%, which supports the theory of the "double peak". Feeding also decreased the systemic availability from 70% when fasted to 45% when fed (van Duijkernen et al., 1996).

In ruminants, age and diet can markedly affect trimethoprim and oral sulfadiazine disposition in calves (Guard et al., 1986; Shoaf et al., 1987). Orally administered sulfadiazine (30 mg/kg) was absorbed very slowly in those calves fed milk diets, with absorption slightly higher in ruminating calves. Trimethoprim was absorbed in preruminant calves, but not absorbed in mature ruminants after oral administration (Shoaf et al., 1987), probably because of inactivation in the rumen.

Sulfasalazine is not used for the antibacterial properties, but is used to treat inflammatory disease of the large intestine in small animals (discussed in more detail in Chapter 46). It is not absorbed as a whole molecule but rather is cleaved into two more active compounds by native resident colonic bacteria.

Distribution

Sulfonamides distribute to most body fluids, but are not distributed to tissues as extensively as trimethoprim. Generally, sulfonamide tissue concentrations are lower than plasma concentrations (approximately 20–30% of corresponding tissue concentration), but distribution to extracellular fluids is generally high enough to produce effective concentrations against susceptible pathogens. High protein binding affects the distribution and markedly increases the half-life of sulfonamides.

Sulfonamides are weak acids and trimethoprim is a weak base (Table 32.1). The ionization affects distribution, which favors the distribution and ion trapping of trimethoprim in tissues (intracellular environment is typically more negative than plasma). Therefore trimethoprim has a higher volume of distribution than sulfonamides. Also, because sulfonamides are weak acids, the pH-partition hypothesis shows that these drugs do not attain therapeutic concentrations in milk; however, enough passive diffusion occurs to limit their use in dairy cattle.

The prostate is another example in which pH-dependent distribution is known to occur (Robb et al., 1971). Sulfadiazine being a weak acid, penetrated the prostate to approximately 11% that of the mean plasma concentration. Because trimethoprim is a weak base (pK_a of 7.3) the concentrations in the prostate are higher owing to ion trapping. The concentration in the prostatic

Table 32.2 Some pharmacokinetic parameters of sulfamethazine (sulfadimidine) in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$t^{1}/_{2}$ (h)	Clearance (ml/h/kg)	Reference
Cattle	107	IV	0.346	NR	NR	Bevill et al., 1977a
Cattle (male)	200	IV	0.37	5.82	45	Witcamp et al., 1992
Cattle (female)	200	IV	0.24	3.64	54	Witcamp et al., 1992
Calves (62–70 days old)	10	IV	NR	5.2	NR	Nouws et al., 1988c
Calves (68–76 days old)	100	IV	NR	5.7	NR	Nouws et al., 1988c
Cows (4–5 years old)	10	IV	NR	4	NR	Nouws et al., 1988c
Cows (3–5 years old)	100	IV	NR	5.9	NR	Nouws et al., 1988c
Cows (5–6 years old)	200	IV	NR	5.5	NR	Nouws et al., 1988c
Pigs (9 weeks old)	50	IV	0.51	16	21	Sweeney et al., 1993
Pigs (10 weeks old)	20	IV	0.604	10	42	Nouws et al., 1989a
Pigs (10 weeks old, given in drench)	20	PO	NR	11.9	NR	Nouws et al., 1989a
Pigs (10 weeks old, given in medicated feed)	20	PO	NR	16.6	NR	Nouws et al., 1989a
Pigs (male, 18–32 kg)	20	IV	0.55	12.4	25	Nouws et al., 1989a
Gilts (12–13 weeks old)	107.5	IA	0.493	15.61	NR	Duffee et al., 1984
Barrows (12–13 weeks old)	107.5	IA	0.614	17.7	NR	Duffee et al., 1984
Boars (12–13 weeks old)	107.5	IA	0.542	16.63	NR	Duffee et al., 1984
Pigs (normal castrated males and intact females)	50	IV	0.50	15	23	Yuan et al., 1997
Pigs (castrated males and intact females infected with *S. suum*)	50	IV	0.52	20	17	Yuan et al., 1997
Goat	100	IV	0.316	2.77	81	Elsheikh et al., 1991
Goats (adult and fed)	100	IV	0.9	4.75	135.6	Abdullah and Baggot, 1988
Goats (adult and fasted)	100	IV	0.897	7.03	69.6	Abdullah and Baggot, 1988
Goats (adult male)	20	IV	0.28	8.7	20	Witcamp et al., 1992
Goats (adult female)	20	IV	0.18	2.13	70	Witcamp et al., 1992
Goats (12 weeks old)	100	IV	0.43	1.97	134	Nouws et al., 1989b
Goats (18 weeks old)	100	IV	0.507	2.56	106	Nouws et al., 1989b
Sheep	100	IV	0.297	4.72	44.6	Elsheikh et al., 1991
Sheep (male)	100	IV	0.4	4.5	90	Srivastava and Rampal, 1990
Ewes	100	IV	0.474	9.51	35.07	Youssef et al., 1981
Ewes (dosed in summer months)	100	IV	0.37	3.64	63	Nawaz and Nawaz, 1983
Ewes (dosed in winter months)	100	IV	0.49	3.92	85	Nawaz and Nawaz, 1983
Sheep (ewes and rams)	100	IV	0.41	10.8	41	Bulgin et al., 1991
Sheep (ewes and rams)	100	PO	NR	4.3	NR	Bulgin et al., 1991
Sheep (ewes and rams)	391	PO	NR	14.3	NR	Bulgin et al., 1991
Sheep (ewes and rams)	100	IV	0.37	3.64	NR	Bulgin et al., 1991
Sheep (ewes and rams)	107.5	IV	0.293	5.87	NR	Bulgin et al., 1991
Sheep (ewes and rams)	107.5	IV	0.327	7.09	NR	Bulgin et al., 1991
Ponies (breed unknown)	160	IV	0.63	11.4	42.1	Wilson et al., 1989
Ponies (Shetland)	20	IV	0.33	5.4	55.2	Nouws et al., 1987
Mare (2 years old)	20	IV	0.47	5	65	Nouws et al., 1985a
Mare (2 years old)	200	IV	0.56	6	67	Nouws et al., 1985a
Mare (22 years old)	20	IV	0.38	9.5	28	Nouws et al., 1985a
Mare (22 years old)	200	IV	0.36	14.6	27	Nouws et al., 1985a
Stallion (1.5 years old)	20	IV	0.44	9.5	32	Nouws et al., 1985a
Stallion (1.5 years old)	200	IV	0.65	11	41	Nouws et al., 1985a
Horse	20	IV	0.33	5.4	54	Nouws et al., 1987
Horse	160	IV	0.63	11.4	48	Wilson et al., 1989
Horse	60	IV	0.74	9.8	NR	
Dogs (normal)	100	IV	0.628	16.2	22.4	Riffat et al., 1982
Dogs (febrile)	100	IV	0.495	16.7	20.2	Riffat et al., 1982
Rabbits (male)	35	IV	0.42	0.4	73.6	Witcamp et al., 1992
Rabbits (female)	35	IV	0.23	0.39	40.8	Witcamp et al., 1992
Carp (10°C)	100	IV	1.15	50.3	16.14	van Ginneken et al., 1991
Carp (20°C)	100	IV	0.9	25.6	24.66	van Ginneken et al., 1991
Rainbow trout (10°C)	100	IV	1.2	20.6	41.1	van Ginneken et al., 1991
Rainbow trout (20°C)	100	IV	0.83	14.7	39.9	van Ginneken et al., 1991
Camel	50	IV	0.73	13.2	40	Younan et al., 1989
Camel	100	IV	0.394	7.36	40.9	Elsheikh et al., 1991
Buffalo (female)	200	IV	1.23	12.36	193.2	Singh et al., 1988

NR, not reported; IV, intravenously; IA, intraarterially; PO, orally; Vd (volume of distribution); $t^{1}/_{2}$ (half-life).

Table 32.3 Some pharmacokinetic parameters of sulfadiazine in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T^{1}/_{2}$ (h)	Clearance (ml/h/kg)	Reference
Pigs	25/5[a]	PO	NR	3.1–4.31	NR	Soli et al., 1990
Pigs	20	IV	0.54	4.0[b]	140	Nielsen and Gyrd-Hansen, 1994
Pigs (fed)	40	PO	NR	11.5[b]	NR	Nielsen and Gyrd-Hansen, 1994
Pigs (fasted)	40	PO	NR	8.1[b]	NR	Nielsen and Gyrd-Hansen, 1994
Carp (10°C)	100/20[a]	IV	0.53	47.1	7.9	Nouws et al., 1993
Carp (20°C)	100/20[a]	IV	0.60	33	12.2	Nouws et al., 1993
Ewes	100	IV	0.39	37.15	38.75	Youssef et al., 1981
Dogs	100/20[a]	PO	NR	9.84	NR	Sigel et al., 1981
Calves (milk diet, 7 weeks)	25/5[a]	SC	NR	3.4	NR	Shoaf et al., 1987
Calves (milk diet, 13 weeks)	25/5[a]	SC	SC	3.4	NR	Shoaf et al., 1987
Calves (grain diet, 7 weeks)	25/5[a]	SC	NR	4.4	NR	Shoaf et al., 1987
Calves (grain diet, 13 weeks)	25/5[a]	SC	NR	3.6	NR	Shoaf et al., 1987
Calves (8–20 days)	20	IV	NR	6.2	NR	Nouws et al., 1988c
Calves (0.5 years)	100	IV	NR	7	NR	Nouws et al., 1988c
Cattle (5 years)	10	IV	NR	4.1	NR	Nouws et al., 1988c
Calves (male, 1 day)	25/5[a]	IV	0.72	5.78	5.8	Shoaf et al., 1989
Calves (male, 7 days)	25/5[a]	IV	0.67	4.4	102	Shoaf et al., 1989
Calves (male, 42 days)	25/5[a]	IV	0.59	3.6	112.8	Shoaf et al., 1989
Calves (7 days, with synovitis)	25/5[a]	IV	28.7	24.44	102	Shoaf et al., 1986
Horses (adult)	20/4[a]	PO	NR	7.8	NR	FOI summary (FDA)
Horses	12.5	IV	0.52	2.7	NR	Brown et al., 1983
Horses	20	IV	0.4	3.8	138	Nouws et al., 1987
Horses	25	PO	NR	7.4	NR	Sigel et al., 1981
Horses (adult)	25	IV	0.58	5.37	100	Winther et al., 2011
Horses (adult)	25	PO (paste, fed)	NR	14.03	NR	Winther et al., 2011
Horses (adult)	25	PO (intragastric, fed)	NR	12.3	NR	Winther et al., 2011
Ponies	25	PO	NR	12.08	NR	Van Duijkeren et al., 2002
Horses (adult)	12.5	IV	0.50	4.6	90	Gustafsson et al., 1999
Horses (adult)	25	PO (fed)	NR	8.2	NR	Gustafsson et al., 1999
Horse (adult)	25	PO (fasted)	NR	8.15	NR	Van Duijkeren et al., 1994c
Horse (adult)	25	IV	0.58	4.65	115.2	Van Duijkeren et al., 1994c

NR, not reported; IV, intravenously; PO, orally; SC, subcutaneously; Vd (volume of distribution); $T^{1}/_{2}$ (half-life).

[a] Sulfadiazine–trimethoprim dose.

[b] Reported as mean residence time (MRT).

fluid has been measured to be 380% higher than that of plasma. Consequently, trimethoprim–sulfonamide combinations are an acceptable choice for treating infections of the prostate.

In horses, studies have been conducted to examine tissue concentrations in urine, peritoneal fluid, endometrium, and synovial fluid (Brown et al., 1983, 1988, 1989) of trimethoprim or ormetoprim–sulfonamide combinations. In each tissue, drug concentrations were adequate for treating infections in these sites. Urine concentrations – as expected because of the route of elimination – were much higher than plasma, but, otherwise, the plasma concentration and tissue concentration curves were parallel. The only tissue in

Table 32.4 Some pharmacokinetic parameters of sulfamethoxazole in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T^{1}/_{2}$ (h)	Clearance (ml/h/kg)	Reference
Horse	2.5	IV	0.301	3.9[a]	90	Peck et al., 2002
Horse	12.5	IV	0.33	3.53	78.2	Brown et al., 1988
Donkey	2.5	IV	0.335	2.7[a]	132	Peck et al., 2002
Mule	2.5	IV	0.337	5.9[a]	60	Peck et al., 2002

NR = not reported; IV = intravenously; SC = subcutaneously; PO = orally; Vd (volume of distribution); $T^{1}/_{2}$ (half-life).

[a] Reported as mean residence time (MRT).

Table 32.5 Some pharmacokinetic parameters of trimethoprim in animals

Species	Dose^a (mg/kg)	Route	Vd (l/kg)	$T^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Cows	8/40	IV	NR	1.18	NR	Davitiyananda and Rasmussen, 1974
Pigs	4	IV	1.8	3.3^b	0.55	Nielsen and Gyrd-Hansen, 1994
Pigs (fed)	8	PO	NR	10.6^b	NR	Nielsen and Gyrd-Hansen, 1994
Pigs (fasted)	8	PO	NR	6.5^b	NR	Nielsen and Gyrd-Hansen, 1994
Calves (male, 1 day old)	5/25	IV	1.67	8.4	2.8	Shoaf et al., 1989
Calves (male, 7 days old)	5/25	IV	2.23	2.11	2.0	Shoaf et al., 1989
Calves (male, 42 days old)	5/25	IV	2.36	0.9	28.9	Shoaf et al., 1989
Calves (7 weeks old, milk diet)	5/25	SC	NR	3.4	126.0	Shoaf et al., 1987
Calves (13 weeks old, milk diet)	5/25	SC	NR	3.4	124.8	Shoaf et al., 1987
Calves (7 weeks old, grain diet)	5/25	SC	SC	4.4	105.6	Shoaf et al., 1987
Calves (13 weeks old, grain diet)	5/25	SC	NR	3.6	112.2	Shoaf et al., 1987
Calves (7 days old)	5/25	IV	28.72	4.44	102.0	Shoaf et al., 1986
Carp (10°C)	20/100	IV	3.1	40.7	47.0	Nouws et al., 1993
Carp (20°C)	20/100	IV	4.0	20.0	141.0	Nouws et al., 1993
Broilers	4/2^c	PO	NR	0.63	NR	Dagorn et al., 1991
Quail (*Coturnix coturnix japonica;* male and female)	10	PO	NR	2.98	NR	Lashev and Mihailov, 1994
Quail (*Coturnix coturnix japonica;* male and female)	4	IV	2.99	2.38	1.129	Lashev and Mihailov, 1994
Pigs	5/25 (Tribrissen 12%)	PO	NR	3.35	NR	Soli et al., 1990
Pigs	5/25 (Trimazin 12%)	PO	NR	4.86	4.86	Soli et al., 1990
Pigs	5/25 (Trimazin Forte 24%)	PO	NR	5.92	NR	Soli et al., 1990
Horses (adult)	4/20^a	PO	NR	3	NR	FOI summary (FDA)
Horse	2.5–8	IV	2	3	720	Van Duijkeren et al., 1994b (mean values from summary of 7 studies)
Horse (adult)	5	IV	2.22	2.43	650	Winther et al., 2011
Horse (adult)	5	PO (paste, fed)	NR	3.33	NR	Winther et al., 2011
Horse (adult)	5	PO (intragastric, fed)	NR	3.2	NR	Winther et al., 2011
Horse (adult)	2.5	IV	1.82	1.5^b	1224	Peck et al., 2002
Donkey	2.5	IV	1.43	1^b	1680	Peck et al., 2002
Mule	2.5	IV	1.35	1.4^b	942	Peck et al., 2002
Horse (adult)	2.5	IV	1.96	2.8	530	Gustafsson et al., 1999
Horse (adult)	5	PO (fed)	NR	5.1	NR	Gustafsson et al., 1999
Horse (adult)	5	IV	1.68	2.74	509.4	Van Duijkeren et al., 1994c
Horse (adult)	5	PO (fasted)	NR	2.58	NR	Van Duijkeren et al., 1994c
Horse (adult)	5	IV	1.51	2.57	463.8	Van Duijkeren et al., 1995
Horse (adult)	25	PO (fasted)	NR	3.11	NR	Van Duijkeren et al., 1995
Horse (adult)	25	PO (mixed with concentrate)	NR	6.46	NR	Van Duijkeren et al., 1995

NR, not reported; IV, intravenously; SC, subcutaneously; PO, orally; Vd (volume of distribution); $T^1/_2$ (half-life).

[a] First dose is trimethoprim; second dose is sulfadiazine (except for Davitiyananda and Rasmussen, 1974, in which the sulfonamide is sulfadoxine).

[b] Reported as mean residence time (MRT).

[c] Dose reported in mg/kg/24 h.

Table 32.6 Some pharmacokinetic parameters of aditoprim, ormetoprim, tetroxoprim, and metioprim in animals

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T^1/_2$ (h)	Clearance (ml/h/kg)	Reference
Aditoprim:						
Calves (80 kg, milk fed)	5.0	IV	10.44	13.0	11.03	Sutter et al., 1993
Calves (80 kg, conventionally fed)	5.0	IV	9.72	14.8	8.20	Sutter et al., 1993
Calves (160 kg, milk fed)	5.0	IV	9.64	10.7	12.17	Sutter et al., 1993
Calves (160 kg, conventionally fed)	5.0	IV	6.29	8.8	10.29	Sutter et al., 1993
Calves (210 kg, conventionally fed)	5.0	IV	7.16	7.2	13.75	Sutter et al., 1993
Calves (80 kg, milk fed)	5.0	PO	NR	11.6	NR	Sutter et al., 1993
Calves (80 kg, conventionally fed)	5.0	PO	NR	11.60	NR	Sutter et al., 1993
Calves (160 kg, milk fed)	5.0	PO	NR	10.2	NR	Sutter et al., 1993
Calves (160 kg, conventionally fed)	5.0	PO	NR	NR	NR	Sutter et al., 1993
Calves (210 kg, conventionally fed)	10.0	PO	NR	16.6	NR	Sutter et al., 1993
Dairy cows (3–7 years old)	5.0	IV	6.28	7.26	820.0	Lohuis et al., 1992
Dairy cows (3–7 years old, mammary endotoxin)	5.0	IV	12.25	about 7 h	1000.0	Lohuis et al., 1992
Horses	5	IV	7.8	12	300	Fellenberg et al., 1990
Ormetoprim:						
Calves (6–8 months old)	5.5/27.5[a]	IV	1.450	1.37	13.71	Wilson et al., 1987
Mare[b]	9.2/45.8[a]	IV	1.66	1.19	671.0	Brown et al., 1989
Tetroxoprim:						
Dogs	5.0	IV	NR	5.45	NR	Vergin et al., 1984
Metioprim:						
Dogs	5.0	IV	NR	3.07	NR	Vergin et al., 1984

NR, not reported; IV, intravenously; PO, orally; Vd (volume of distribution); $T^1/_2$ (half-life).

[a] First dose is trimethoprim; second dose is sulfadimethoxine.

[b] One mare studied.

which drug concentrations are low is the central nervous system (Brown et al., 1988). Although trimethoprim–sulfonamides can be used to treat CNS infections, higher doses may be required in order to reach effective concentrations (Brown et al., 1988). Administration of dimethylsulfoxide (DMSO) concurrently does not increase the penetration across the blood–brain barrier (Green et al., 1990).

Metabolism

Metabolism and elimination have been examined in several of the veterinary species. One phenomenon that is apparent from these studies is that herbivores metabolize sulfonamides and trimethoprim at a faster rate and more extensively than carnivores or omnivores. This may be caused by a higher metabolic capacity among herbivores – because of the nature of their diet and compounds to which they are exposed – compared to carnivores. Metabolic pathways are discussed in more detail by Nouws et al. (1988c, 1987). Acetylation of the NH_2 group on N-4 is a major mechanism of metabolism. Hydroxylation of the methyl group on the pyrimidine ring, in addition to carboxylation, also occurs. The extent to which these metabolites are produced is drug- and species-dependent. Acetylation and hydroxylation increases the polarity of the sulfonamides, which increases excretion (Nouws et al., 1988c). Acetylation (mainly occurring in the liver and lung) is the major pathway by which sulfonamides are metabolized in most species. Acetylated metabolites are the major urinary metabolites in cattle, sheep, and swine. The canine (dogs and other canine species) lacks the ability to acetylate aromatic amines, relying on alternative metabolic pathways to convert sulfonamides to less active forms. Acetylated metabolites are less soluble than the parent compounds and increase the risks of renal tubular injury caused by precipitation and crystal formation. Glucuronide conjugation and aromatic hydroxylation are two additional metabolic pathways by which sulfonamides are metabolized in animals. Glucuronide metabolites are water soluble and are excreted in urine, decreasing the risk of precipitation in renal tubules. Deacetylation, oxidation, deamination, conjugation with sulfate, and cleavage of heterocyclic rings of sulfonamide molecules have also been reported (Bevill, 1988). Regardless of the metabolic pathway taken, metabolites have either reduced therapeutic activity (hydroxy metabolite) or are therapeutically inactive (N4-acetyl metabolite).

Excretion

Sulfonamides that are capable of obtaining therapeutic blood concentrations are excreted by the kidneys, either as the parent compound or as metabolites via glomerular filtration (unbound to plasma proteins). Subsequently, urine concentrations are consistently higher than the corresponding plasma drug concentrations (approximately 10 times higher for trimethoprim and 30 times higher for the sulfonamides), which aids in the treatment of lower urinary tract infections. There also is some active carrier-mediated proximal tubular excretion and passive absorption of the nonionized drug from the distal tubular fluid. Small concentrations of sulfonamides are also excreted in the tears, feces, bile, milk, and sweat. Low urine pHs favor tubular reabsorption and hence longer half-lives of the sulfonamides, whereas alkalinization of the urine increases urinary excretion by slowing this pH-dependent passive reabsorption in the tubules. Tubular reabsorption is responsible for the long half-lives observed for some sulfonamides. Nonabsorbed sulfonamides intended for intestinal activity are primarily eliminated via the feces, with little of the active or metabolized drug being absorbed systemically to be excreted by these renal mechanisms.

Adverse Effects Caused by Sulfonamides

Sulfonamides can produce a variety of adverse effects in animals. Likewise, when trimethoprim- or ormetoprim–sulfonamide combinations are administered, the adverse effects are primarily attributed to the sulfonamide component.

Crystalluria

Crystalluria, hematuria, and renal tubule blockage can occur owing to precipitation of the sulfonamide in the glomerular filtrate of the kidney. Subsequently, crystals of sulfonamides can form in the renal tubules. This problem is not as important as it once was because it was caused primarily from older insoluble preparations. Sulfadiazine is the least soluble and can precipitate in renal tubules at acidic pH. Even though this complication is rare with the current use of sulfonamides, one should ensure that patients are well hydrated when receiving sulfonamides because renal failure caused by sulfonamide crystals has been reported in human patients that are dehydrated. The tubular blockage has been anecdotally reported in animals, but with current formulations it is not considered an important clinical problem. It is noteworthy that this problem is more likely with acetylated metabolites of sulfonamides, which are not formed in dogs.

Keratoconjunctivitis Sicca

Keratoconjunctivitis sicca (KCS), also known as “dry eye”, is a lack of adequate tear production resulting in ocular inflammation, irritation, and susceptibility to infection. Several cases of sulfonamide-induced KCS have been reported in dogs treated with sulfasalazine, sulfadiazine, and sulfamethoxazole (Morgan and Bachrach, 1982; Slatter and Blogg, 1978; Collins et al., 1986). The reaction is seen most commonly after chronic treatment, but cases have been reported that received only short-term administration. Berger et al. (1995) observed 33 dogs of various breeds for the occurrence of KCS after trimethoprim–sulfadiazine treatment, as characterized by changes in the Schirmer tear test values. There has been disagreement as to whether this effect is caused by an intrinsic dose-related effect, or is idiosyncratic. The prognosis appears to depend on the animal’s age and duration of exposure (Morgan and Bachrach, 1982). Dogs treated with sulfonamides should have tear production checked periodically.

The reaction apparently is caused by a lacrimotoxic effect of the sulfonamide component (toxic to the lacrimal acinar cells). The lacrimotoxic effect may be caused by the nitrogen-containing pyridine ring on the lacrimal acinar cells (Collins et al., 1986; Slatter and Blogg, 1978). Reversal of KCS may or may not occur once sulfonamide therapy has been discontinued.

Hypersensitivity

A delayed hypersensitivity reaction has been described primarily in dogs (Trepanier, 1999; Trepanier et al., 2003). The reaction may be caused by either sulfadiazine, sulfadimethoxine, or sulfamethoxazole. Doberman Pinschers may be more susceptible than other breeds (Giger et al., 1985; Cribb, 1989; Cribb and Spielberg, 1990). This may be a serum sickness reaction (type III hypersensitivity) or involve another mechanism of cytotoxicity and hypersensitivity, or may be idiosyncratic (Trepanier, 2004). The lesions include, but are not limited to, glomerulopathy, polymyositis, polyarthritis, skin rash, skin eruptions, fever, hepatotoxicity, thrombocytopenia, neutropenia, and anemia (Giger et al., 1985; Cribb, 1989; Rowland et al., 1992). The reaction is caused by the sulfonamide component rather than trimethoprim (Giger et al., 1985). In affected dogs, the clinical signs quickly resolved after the sulfonamide was discontinued. However, some problems such as hepatopathy did not resolve in dogs after the initial drug-induced injury (Trepanier et al., 2003). There is some evidence that a reaction to a metabolite of the sulfonamide, rather than an immunological reaction to the parent drug is responsible for these signs (see Section Effect of Acetylator Status on Adverse Reactions).

Hepatic Necrosis

A component of the hypersensitivity reaction is hepatic necrosis. Trimethoprim–sulfadiazine and trimethoprim–sulfamethoxazole combination therapy in dogs has resulted in hepatic necrosis (Twedt et al., 1997; Dodds, 1997). Hepatotoxicity may be caused by a hypersensitivity reaction or a result of an abnormal metabolic pathway, which allows the production or accumulation of hepatotoxic metabolites.

Blood Clotting Disorders

Hypoprothrombinemia has been reported in dogs (Neer and Savant, 1992; Patterson and Grenn, 1975), in coyote pups (Brown et al., 1982), and in Leghorn chickens (Daft et al., 1989) given sulfaquinoxaline. Sulfaquinoxaline is unique among the sulfonamides in that it can induce hypoprothrombinemia in animals within 24 hours after dosing by lengthening prothrombin times. It is thought that this adverse effect is unrelated to the individual sulfonamide or to the quinoxaline portion of the sulfaquinoxaline molecule but occurs when the two entities are combined into a single molecule. Sulfaquinoxaline is not an anticoagulant in vitro, nor does it destroy or otherwise inactivate prothrombin. Nevertheless, sulfaquinoxaline can be an inhibitor of vitamin K epoxide reductase, and this inhibition is the most likely reason for the hypothrombinemic reaction seen in the reported cases of sulfaquinoxaline toxicosis. Treatment is by vitamin K_1 administration for 4–7 days, and recovery is usually uneventful.

Blood Dyscrasias

Anemia and thrombocytopenia have been associated with administration of sulfonamides (Weiss and Adams, 1987; Weiss and Klausner, 1990; Stockner, 1993). Mammals derive their folic acid preformed, either in the diet or from bacteria that produce the vitamin in the intestinal tract. The anemia induced by trimethoprim–sulfonamide combinations may be caused by decreased serum folate reductions, presumably by inhibiting the folate production by intestinal bacteria or by blocking its reduction to tetra- and dihydrofolate, resulting in lowered serum folate concentrations in the animal's serum, which eventually induce an anemia. Folate deficiency anemia is rare but should be monitored with long-term use. Some veterinarians administer folic acid or folinic acid (vitamin B supplements) to patients receiving trimethoprim–sulfonamides. Whether this is routinely necessary, or whether this is effective, is controversial and unproven.

Thrombocytopenia has been reported in animals and in humans (Sullivan et al., 1992; Dodds, 1993). The thrombocytopenia in animals, as in humans, is probably associated with an immune-mediated or hypersensitivity reaction. The thrombocytopenia usually resolves after the drug is discontinued.

Thyroid Metabolism Disorders

Both sulfamethoxazole and sulfadiazine have been associated with hypothyroidism in dogs. The effect is probably caused by the ability of sulfonamides to inhibit thyroid peroxidase activity. Studies have demonstrated that administration of trimethoprim–sulfamethoxazole at a dose of 30 mg/kg, q 12 h for 6 weeks, or 15 mg/kg q 12 h for as short as 3 weeks decreased thyroxine (T_4) levels in dogs and decreased thyroid stimulating hormone (TSH) response (Frank et al., 2005; Hall et al., 1993; Williamson et al., 2002). The hypothyroidism is reversible, and can return to normal in as short as 1 week, or in most dogs by 3 weeks, after discontinuing the drug (Hall et al., 1993; Williamson et al., 2002). One study (Panciera and Post, 1992) produced conflicting evidence in which administration of trimethoprim–sulfadiazine at a dose of 15 mg/kg, q 12 h for 4 weeks had no effect on total T_4, free-T_4, or TSH tests. Sulfadimethoxine has also been implicated as being goitrogenic to swine fetuses in late gestation (Blackwell et al., 1989).

Skin Reactions

Sulfonamides are among the most common drugs implicated in skin eruptions in people, especially the drug-induced Stevens–Johnson syndrome and toxic epidermal necrolysis (Roujeau et al., 1995). In dogs, skin reactions (drug eruptions) also are possible (Medleau et al., 1990). The skin reactions in dogs are believed to be a manifestation of the hypersensitivity reaction described in Section Hypersensitivity (Trepanier, 1999).

Effect of Acetylator Status on Adverse Reactions

Adverse effects in people have been associated with acetylator status. In slow acetylators a greater proportion of the drug may be directed to conversion of a more toxic metabolite, sulfonamide–hydroxylamine or nitroso compounds, which are more toxic to cells. Ordinarily, these metabolites are detoxified by glutathione conjugation, but some patients may lack this ability. Dogs lack ability to acetylate drugs; therefore, they may be more susceptible to adverse effects than other animals. There is evidence that some dogs are more susceptible to the adverse effects of the hydroxylamine–sulfonamide metabolite than mixed-breed dogs because of decreased ability to handle the metabolite, which may explain the higher incidence of adverse effects reported in Dobermans (Cribb and Spielberg, 1990). (Other drugs that are acetylated in people include dapsone and isoniazid and these also may present a higher risk of toxicity in dogs.)

Diarrhea

Diarrhea has been associated with trimethoprim–sulfonamide therapy in horses. However, this effect may not be any more common from trimethoprim–sulfonamides than from other orally administered antimicrobials in horses. When healthy horses were administered doses of 25 to 100 mg of sulfadiazine in combination with 5 to 20 mg/kg of trimethoprim there was no evidence of increased *Clostridium perfringens*–associated colitis (White and Prior, 1982). In another study, Gustafsson et al. (1999) administered trimethoprim–sulfadiazine twice daily for 5 days to horses and measured the effect on intestinal flora. There was an initial decline in bacterial numbers, but these rebounded and the authors concluded that this treatment did not produce a disturbance of the intestinal bacterial flora.

Carcinogenesis

Sulfamethazine has been demonstrated to induce thyroid hyperplasia in rats (Astwood et al., 1943; MacKenzie and MacKenzie, 1943; Swarm et al., 1973) and induce specific types of thyroid cancer in both mice and rats. Fullerton et al. (1987) found that male and female Fischer 344 rats fed diets containing 1200 or 2400 ppm of sulfamethazine had thyroid weights that were increased significantly more than controls and that these increased weights were most likely due to thyroid hyperplasia related to increased thyroid-stimulating hormone levels. Littlefield et al. (1989) fed sulfamethazine to mice and induced follicular cell adenomas of the thyroid gland after 24 months of continuous feeding at a 4800 ppm dose with focal follicular cell hyperplasia and other organ aberrations being noted at some of the lower doses of sulfamethazine. In a similar study, there was a statistically significant increase in the incidence of thyroid follicular cell adenocarcinomas in rats sacrificed after 24 months of continuous feeding of sulfamethazine, with other nonneoplastic lesions of the thyroid also being reported in other treatment groups. There are no reports that have associated sulfonamide administration with cancer in domestic animals.

Potassium Regulation

Trimethoprim has been associated with hyperkalemia in people and laboratory animals, but except for anecdotal accounts, this has not been well documented in veterinary species. The mechanism of hyperkalemia appears to be caused by inhibition of renal Na-K-ATPase in the face of intact H-K-ATPase activity. The effects of trimethoprim can mimic amiloride, a potassium-sparing diuretic. These effects could be potentiated by coadministration of angiotensin converting enzyme inhibitors (ACE inhibitors), such as enalapril or benazepril, or administration of an angiotensin receptor blocker.

Sulfonamides in Veterinary Medicine

Sulfadimethoxine

Sulfadimethoxine (Figure 32.1) is a long-acting sulfonamide that has been used alone or in combination with ormetoprim (ormetoprim–sulfadimethoxine) for the treatment of susceptible microbial infections of cattle, swine, horses, poultry, fish, and dogs, in addition to other vertebrate and invertebrate animals. The combination is discussed in Section Sulfadimethoxine–Ormetoprim.

Sulfadimethoxine pharmacokinetics have been reported for many species. In dogs, the oral absorption is 49%, with a half-life of 13.1 hours (Baggot et al., 1976). Peak serum concentrations in dogs, at a dose of 55 mg/kg oral, was 67 μg/ml (mean). Systemic clearance in dogs is via the kidneys.

Sulfadimethoxine pharmacokinetics in cattle has been described by many investigators. Bourne et al. (1981) administered adult cattle with 107 mg/kg either intravenously (IV) or orally. In the IV study, sulfadimethoxine plasma concentrations peaked at 0.5 hours after administration and slowly declined over time, with the parent compound, acetylsulfadimethoxine, and a “polar” metabolite being found in the urine for at least 48 hours after the IV dose. The volume of distribution (Vd) was 0.315 l/kg in those cattle. In the oral study, plasma concentrations of sulfadimethoxine started low at 0.5 hours and gradually peaked at 10 hours after dose and then began to drop, with detectable levels of parent compound and all metabolites being found in the urine for at least 84 hours after dosing. Bioavailability of sulfadimethoxine was calculated to be 59.1%. Boxenbaum et al. (1977) administered 55 mg/kg IV sulfadimethoxine (40% solution) or 55 mg/kg orally to cattle, followed by 27.5 mg/kg sulfadimethoxine administered orally at

24, 48, and 72 hours after the initial loading dose. After IV injection, the half-life of sulfadimethoxine was determined to be 12.5 hours, with a volume of distribution of 0.31 l/kg. This study also confirmed that adequate plasma concentrations (>50 μg/ml) were maintained throughout the oral-dosing study, and this method could be used when IV administration was not possible. By comparison, a study by Wilson et al. (1987) demonstrated that sulfadimethoxine (27.5 mg/kg) in combination with ormetoprim (5.5 mg/kg) administered IV to cattle had a shorter half-life, 7.91 hours, and volume of distribution of 0.185 l/kg. When given the same dose orally, bioavailability of sulfadimethoxine was 56.6%.

Studies by Righter et al. (1979) examined the pharmacokinetics of sulfadimethoxine in mature, growing, and suckling pigs. Mature pigs dosed with 20, 50, or 100 mg/kg of sulfadimethoxine IV had volume of distribution values of 0.178, 0.258, and 0.331 l/kg and total body clearance of 4.21, 5.54, and 7.37 ml/kg/h, respectively. The pharmacokinetic parameters of 55 mg/kg sulfadimethoxine given IV to growing and suckling pigs have also been reported. Suckling pigs (1–2 weeks old) had sulfadimethoxine half-lives of 16.16 hours, volume of distribution of 0.483 l/kg, and total body clearance of 20.9 ml/kg/h. In contrast, growing pigs (11–12 weeks old) had sulfadimethoxine half-lives of 9.35 hours, volume of distribution of 0.347 l/kg, and total body clearance of 26.1 ml/kg/h, indicating an age-related effect of sulfadimethoxine pharmacokinetics in young pigs. Weanling pigs consuming water dosed with 0.05 g sulfadimethoxine/100 ml showed mean plasma concentrations of 80 ppm 12 hours after introduction of the medicated water, with plasma concentrations declining to approximately 50 ppm thereafter. Total water consumption was not affected, which indicated that sulfadimethoxine may be of therapeutic use in swine provided that water consumption is maintained throughout the medication period. Mengelers et al. (1995) dosed 34–40 kg healthy and febrile (inoculated endobronchially with *Actinobacillus pleuropneumoniae* toxins) pigs with 25 mg/kg sulfadimethoxine and 5 mg/kg trimethoprim intravenously. Sulfadimethoxine plasma half-lives for both healthy and pneumonic pigs were not significantly different (approximately 13 hours). Trimethoprim half-lives were not significantly different between healthy and pneumonic pigs (approximately 2.7 hours); however, the half-lives were significantly shorter than the half-life of sulfadimethoxine. In addition, the volume of distribution values of healthy and pneumonic pigs receiving sulfadimethoxine were not significantly different (approximately 0.25 l/kg), but trimethoprim did show significant differences between healthy (1.21 l/kg) and pneumonic (1.49 l/kg) pigs. The mean area under the curve (AUC) of trimethoprim was decreased and the total body clearance was increased in the febrile pigs, but with no significant changes in these sulfadimethoxine pharmacokinetic parameters.

Sulfadimethoxine is available in a concentrated solution (e.g., Albon 12.5%) for cattle or poultry; oral suspension (e.g., 5% Albon Suspension), and tablets and boluses for dogs, cats, and cattle; extended release tablets (Albon SR); 40% injection used in dogs, cats, and cattle; and soluble powder that can be added to drinking water for cattle and poultry. The approved clinical use for this formulation is for treatment of intestinal coccidiosis, bacterial enteritis, fowl cholera, bacterial pneumonia, pododermatitis in cattle, skin and soft tissue infections in dogs and cats, and bacterial cystitis in dogs. On the product labels for treating bacterial infections, it states "for treatment of susceptible bacteria causing these infections." Because resistance can be common, some of the conditions listed above may not respond appropriately to treatment and may be outdated.

The clinical use of sulfadimethoxine has been described for turkeys (Epstein and Ashworth, 1989), dogs (Yagi et al., 1981; Fish et al., 1965; Dunbar and Foreyt, 1985; Imamura et al., 1986, 1989), primates (Adamson et al., 1970; Bridges et al., 1968), lobsters (James and Barron, 1988), channel catfish (Squibb et al., 1988), and rainbow trout (Kleinow and Lech, 1988). Detection of violative levels of sulfadimethoxine residues in channel catfish has also been reported (Walker and Barker, 1994).

Sulfamethazine (Sulfadimidine)

Sulfamethazine (sulfadimidine) (Figure 32.1), like many sulfonamides, has been utilized for decades in veterinary medicine; hence, the veterinary literature contains many reports on its usage in a wide variety of animals, including cattle, horses, swine, poultry, small ruminants, and rabbits (among others). Table 32.2 summarizes some of the pharmacokinetic parameters of sulfamethazine in animals.

Sulfamethazine has been administered to cattle and swine, and is formulated for use in drinking water (Church et al., 1979), as a feed additive, an extended-release bolus, and an IV preparation. Sulfamethazine has been marketed as a sole treatment and in combination with other antimicrobials, such as other sulfonamides, tylosin, chlortetracycline, and procaine penicillin G.

The product labels for sulfamethazine products include uses for treating intestinal coccidiosis, bacterial enteritis, pneumonia, and pododermatitis in cattle. Sulfamethazine is available as an oral solution for calves, poultry, pigs, and cattle. It is available as a 12.5% solution to be added to drinking water. It is also available as a powder to be added to the drinking water of these species. Tablets (Sulmet 2.5 and 5-gram tablets) are also available for large animals, as well as extended-release tablets (e.g., Sulfa-Max) in a range of sizes for calves and adult cattle. Triple-sulfa products containing sulfamethazine (sulfamethazine, sulfanilamide, and sulfathiazole) as well as sulfamethazine–sulfathiazole combination oral powder are no longer available in the United States, but may still be available in other countries.

The basic pharmacokinetic parameters of sulfamethazine in cattle have been reported by Bevill et al. (1977a) and Nouws et al. (1988c), among many others. Of particular interest are the oral forms of sulfamethazine that have been formulated in extended-release (sustained-release) form for cattle. Several reports on the efficacy and clinical uses of the extended-release form of sulfamethazine in cattle are available (Clark et al., 1966; Ellison et al., 1967; Miller et al., 1969; Carlson et al., 1976). This sustained-release formulation has been reported to achieve blood concentrations sufficient for susceptible bacteria within 6–12 hours after oral administration and to maintain or exceed that level for 2–5 days after dosing. The sustained-release formulation of sulfamethazine has been reported to be effective for treating bovine respiratory disease, diphtheria, and pneumonia in cattle (Carlson et al., 1976; Clark et al., 1966). Clearance of sulfamethazine and its metabolites in cattle are age and dose dependent (Nouws et al., 1986a, 1985b, 1983; Lapka et al., 1980). Several metabolites of sulfamethazine have been identified and described in both adult cattle and calves (Nouws et al., 1988c).

The pharmacokinetics of sulfamethazine and its metabolites are of particular interest in swine. Sulfamethazine and its metabolites were often associated with violative levels in pork products because of sulfamethazine's widespread use as a swine feed additive. Sulfamethazine has been used extensively to treat a host of susceptible microbial infections in swine, including *Salmonella typhisuis* (Fenwick and Olander, 1987) and *Bordetella bronchiseptica* (Kobland et al., 1984). Sulfonamides were one of the most common causes of food-residue violations reported by the US Food Safety Inspection Service, with swine being the food-animal species with the greatest number of residue violations (Sweeney et al., 1993).

Pharmacokinetic parameters for swine have been described by Sweeney et al. (1993) and others (see Table 32.2), including pharmacokinetics of metabolites (Nouws et al., 1989a, 1986b). Several studies have used radiolabeled (Mitchell et al., 1986; Mitchell and Paulson, 1986) and nonradiolabeled (Biehl et al., 1981; Ashworth et al., 1986) sulfamethazine to determine the elimination patterns of sulfamethazine and its metabolites from the tissues in swine. Other studies have shown that the major metabolites produced from sulfamethazine metabolism in swine are N_4-acetylsulfamethazine, N_4-glucose conjugate of sulfamethazine, and desaminosulfamethazine (Mitchell et al., 1986). Studies using pigs fed 110 ppm of ^{14}C-sulfamethazine in the feed for 3–7 days, euthanized, and their tissues examined for total radioactivity and metabolite content found the highest concentration of radioactivity in the gut (undigested feed). Blood, kidney, urine, and liver all had high concentrations of radioactivity (i.e., parent compound and metabolite). Adipose tissue contained the least amount of radioactivity of all tissues assayed (Mitchell et al., 1986). Specific metabolites found in these and other tissues of swine given ^{14}C-labeled sulfamethazine in the feed have been reported by Mitchell and Paulson (1986).

Other studies have also reported on sulfamethazine residues in swine (Ashworth et al., 1986; Biehl et al., 1981). In addition, sulfamethazine and its metabolites have been described in pigs using physiological based pharmacokinetic (PBPK) models (Buur et al., 2005, 2006; Mason et al., 2008). These studies demonstrated significant amount of variability in disposition and metabolism, which would suggest potential for tissue residue violations with minor differences in dosing, environmental contamination, or in the presence of disease.

Cattle and swine are the two major species in which sulfamethazine is approved for use, with fewer reports in other species. Pharmacokinetic parameters and/or tissue-depletion kinetics of sulfamethazine and metabolites have been established in ponies (Wilson et al., 1989; Nouws et al., 1987) and horses (Nouws et al., 1985a). Studies on pharmacokinetics of sulfamethazine in goats (Abdullah and Baggot, 1988; Witcamp et al., 1992; Nouws et al., 1988b, 1989b; Elsheikh et al., 1991; Youssef et al., 1981; van Gogh et al., 1984; Witcamp et al., 1993), sheep (Srivastava and Rampal, 1990; Bourne et al., 1977; Bevill et al., 1977c; Bulgin et al., 1991; Nawaz and Nawaz, 1983), dogs (Riffat et al., 1982), chickens (Righter et al., 1971; Nouws et al., 1988a; Goren et al., 1987), rabbits (Yuan and Fung, 1990), mice (Littlefield et al., 1989), buffalo (Singh et al., 1988), camels (Younan et al., 1989), and carp and rainbow trout (van Ginneken et al., 1991) also have been published.

Lashev et al. (1995) described altered pharmacokinetics in roosters treated with a single 50 mg/kg IV dose of sulfadimidine only or IV sulfadimidine after 2 weeks of four 3.5 mg/kg subcutaneous (SC) testosterone treatments. Normal and castrated roosters provided no significant differences in half-life values, which ranged from 7.62 hours (castrated) to 9.38 hours (intact). Roosters pretreated with testosterone and then dosed with sulfadimidine had half-lives of 23.85 hours, as well as significantly decreased clearance and volume of distribution. Chickens metabolize sulfamethazine in relatively equal parts through hydroxylation and acetylation. It was hypothesized in this study that the acetylation pathway of sulfamethazine metabolism was retarded by the testosterone treatments and resulted in the prolonged half-lives.

Sulfaquinoxaline

Sulfaquinoxaline (Figure 32.1) has been used primarily for control of coccidia and some susceptible bacterial diseases in poultry. The veterinary literature also contains a few reports of sulfaquinoxaline use in rabbits (Eppel and Thiessen, 1984; Joyner et al., 1983) and dogs (Brown et al., 1982; Patterson and Grenn, 1975), but clinical use in these species is rare today.

Sulfaquinoxaline is available as an oral solution in a range of concentrations (20–32%). These solutions are intended to be mixed with drinking water. In some countries (but not the US) there are combinations of pyrimethamine and sulfaquinoxaline solution for administration to drinking water.

For the use in poultry, sulfaquinoxaline has been administered to control coccidiosis. Mathis and McDougald (1984) described the therapeutic effectiveness of sulfaquinoxaline and sulfaquinoxaline–pyrimethamine against several coccidia species of *Eimeria*. It was determined from that study that both sulfaquinoxaline and sulfaquinoxaline–pyrimethamine were highly effective against *E. acervulina* but less effective against *E. tenella*. In addition, the potentiated mixture was determined to be more effective against *E. tenella* than sulfaquinoxaline alone, although neither mixture was found to be particularly effective against any cecal coccidia. Amprolium was efficacious against cecal–dwelling forms of coccidia; hence amprolium has been combined with sulfaquinoxaline or sulfaquinoxaline–pyrimethamine to enhance the spectrum of activity. Ineffectiveness of sulfaquinoxaline–pyrimethamine against *E. tenella* has also been documented in another study (Chapman, 1989), underlining the importance of correct coccidia species identification before instituting anticoccidial therapy with sulfaquinoxaline or any other sulfonamide.

Banerjee et al. (1974) reported on the blood concentrations after administration of sulfaquinoxaline, which were in the therapeutic range. In that same study, sulfaquinoxaline was found in high concentrations in the liver, kidney, and cecum, with the lowest concentrations found in the yolk sac and brain. A single oral dose of ^{35}S-labeled sulfaquinoxaline in 1-week-old chicks showed rapid uptake from the gastrointestinal tract and wide distribution throughout the body, including crossing of the blood–brain barrier. At 0.5 hours after dosing, autoradiography showed that all tissues (brain, lung, liver, kidney, fat, and muscle), except the lens of the eye had measurable concentrations of sulfaquinoxaline. Similar findings resulted from IV administration of ^{35}S-labeled sulfaquinoxaline, and it was also found that excretion of sulfaquinoxaline by the bile and secretion by the cecal mucosa, crop, and gizzard probably occur. Oral absorption was higher (3.5 times) in birds infected with *E. acervulina* and *E. tenella* compared to uninfected birds (Williams et al., 1995). A study by Li et al. (1995) found that in 7- to 8-week-old male and female broilers given a single 200 mg/kg oral dose of sulfaquinoxaline, peak concentration times in plasma and liver were similar (4 hours) but were longer in the heart, kidney, and muscle (6 hours). The half-life of sulfaquinoxaline was shortest in the muscle (4.5 hours), with significantly longer half-lives in the heart (10 hours), plasma (11 hours), liver (13 hours), and kidney (18 hours).

The safety and efficacy of sulfaquinoxaline alone or in combination with trimethoprim (trimethoprim:sulfaquinoxaline ratio is 1:3) have been reported in poultry (White and Williams, 1983; Piercy et al., 1984; Sainsbury, 1988). A total dose of 30 mg/kg/day PO satisfactorily controlled experimentally induced colisepticemia and pasteurellosis in addition to five species of coccidia (White and Williams, 1983). A wide margin of safety has been shown for the 1:3 combination of trimethoprim:sulfaquinoxaline in poultry, although decreased appetite and water consumption and lowered egg production, egg weight, and hatchability were noted when these antimicrobials were incorporated in the feed or water in higher than recommended concentrations (Piercy et al., 1984).

Toxicosis from sulfaquinoxaline use in animals is rare. Toxicity from sulfaquinoxaline has occurred in Leghorn chickens (Daft et al., 1989), where a mortality of 47% was reported in a commercial flock given a 0.05% concentration of sulfaquinoxaline in the feed. Lesions included mildly enlarged livers; swollen and pale livers; hemorrhages on the epicardium, kidney, oviduct, small intestine, and cecum; pale bone marrow; gangrenous dermatitis; and some lung involvement was present. Patterson and Grenn (1975) reported on 12 adult Miniature Poodles that received 3.16 g/l of sulfaquinoxaline in the drinking water as treatment for coccidiosis suffered similar lesions as described above in poultry, in addition to depressed body temperature, pale mucous membranes, microscopic hemorrhages of the jejunum and ileum, and prolonged prothrombin times. Although the exact mechanism has not been reported, sulfaquinoxaline possesses an ability to produce a marked hypothrombinemia (see Section Adverse Effects Caused by Sulfonamides), even in animals receiving balanced diets containing adequate amounts of vitamin K. These animals also responded to vitamin K treatment. It is thought that this adverse effect is not related to the individual sulfonamide or quinoxaline portion of the sulfaquinoxaline molecule but occurs only when the two entities are combined. A similar toxicosis has also been reported in coyote pups treated with sulfaquinoxaline (Brown et al., 1982).

Sulfamerazine

Sulfamerazine (Figure 32.1) has primarily been utilized in adult sheep and lambs to treat susceptible microbial infections. In these ruminants, sulfamerazine has been used alone or in combination with other antibiotics (tylosin) and other sulfonamides (sulfamethazine, sulfadiazine).

The pharmacokinetics of sulfamerazine has been described for ewes and lambs. Hayashi et al. (1979) described the pharmacokinetics of sulfamerazine in ewes after either IV or oral administration of 107 mg/kg. After oral administration, systemic availability was 81 ± 19%. Urinary concentrations of parent compound and metabolites were also reported for both IV and PO dosing studies. Both routes produced appreciable concentrations of sulfamerazine and three metabolites in the urine (described as a polar metabolite acetylsulfamerazine). Intravenous sulfamerazine produced more parent compound and fewer metabolites were found in the urine, which is attributed to lack of rumen metabolism, while more metabolite than parent compound was found in the PO study because of conversion by rumen metabolism.

The pharmacokinetics of sulfamerazine has also been reported in neonatal and young lambs (Debacker et al., 1982). Lambs from birth to 16 weeks of age were administered either IV or PO, 100 mg/kg of sulfamerazine. In the IV study, it was found that the sulfamerazine half-life was longest in the first week of life (9–14 hours) and decreased to 4–7 hours by 9–16 weeks of age. The volume of distribution was highest during the first week of life and steadily decreased with age, while clearance of sulfamerazine was lowest in the first week of life (20–40 ml/kg/h) and steadily increased with age up to 9–16 weeks of age (50–80 ml/kg/h).

Sulfathiazole

Sulfathiazole use in veterinary medicine has declined. It has been formulated in combination with chlortetracycline HCl and procaine penicillin G, but reports of its use are rare and earlier editions of this text should be consulted for more details.

Sulfathiazole pharmacokinetics in sheep has been outlined by Koritz et al. (1977), and sulfathiazole tissue residues in sheep have been described by Bevill et al. (1977b). When 36 or 72 mg/kg of 5% aqueous solution of sulfathiazole sodium IV was given to ewe lambs, it cleared quickly from the plasma, with a low volume of distribution of 0.34 and 0.59 l/kg and half-lives of 1.2 and 1.4 hours, respectively. Ewes given 214 mg/kg orally of a 12.5% aqueous solution of sulfathiazole sodium had systemic bioavailability of approximately 73%, with a half-life of approximately 18 hours. Both oral and IV routes produce acetylsulfathiazole, and a third “polar” metabolite in the urine of these sheep. Sulfathiazole residues in sheep are highest in the kidney (308 ppm), followed by the liver (40 ppm), heart (34 ppm), shoulder muscle (23 ppm), leg and loin muscle (22 ppm), body fat (11 ppm), and omental fat (6.7 ppm). Residues quickly dropped to very low (<0.13 ppm) or to nondetectable levels by 24 hours after dosing in all tissues tested.

Pharmacokinetic parameters have also been reported for swine. Pigs given 72 mg/kg of sulfathiazole sodium IV had quick plasma elimination of the drug, with mean Vd of 0.54 l/kg and a biological half-life of 1.39 hours, similar to those for sheep. After a dose of 214 mg/kg orally, sulfathiazole had a Vd of 0.32 l/kg and a systemic bioavailability of 73%, identical to that of sheep.

Sulfasalazine (Salicylazosulfapyridine)

Sulfasalazine (Figure 32.3) was originally developed as a possible treatment for rheumatoid arthritis in humans. It was found, however, to be more effective in the treatment of inflammatory bowel disease. The most frequent use is to treat various forms of colitis (Aronson and Kirk, 1983). The gastrointestinal use is discussed in more detail in Chapter 46 of this book. Inflammatory bowel diseases (most commonly ulcerative colitis and Crohn’s disease) have been treated with sulfasalazine in humans.

Sulfasalazine consists of two components, 5-aminosalicylic acid and sulfapyridine, which are linked by an azo bond. This bond is broken by the bacterial enzymes in the colon (Figure 32.3). After oral administration, the sulfonamide component is absorbed systemically, but the 5-aminosalicylic acid component produces a local inflammatory effect in the colon. 5-aminosalicylic acid is also known as *mesalamine* and there are now other forms of mesalamine (e.g., olsalazine) or enteric-coated (pH-sensitive) tablets that release mesalamine in the colon, while avoiding the systemic effects of the sulfonamide component.

The intestinal antiinflammatory effects may be due to prostaglandin inhibition (Hoult and Moore, 1978), inhibition of leukotrienes, decreased intestinal oxygen free radicals (Del Soldato et al., 1985), or sulfhydryls (Garg et al., 1991).

Sulfadiazine

The most commonly used formulation containing sulfadiazine is a trimethoprim–sulfadiazine combination (e.g., Tribrissen and others). This combination will be discussed in more detail in Section Trimethoprim–Sulfadiazine, because of its common use. Sulfadiazine

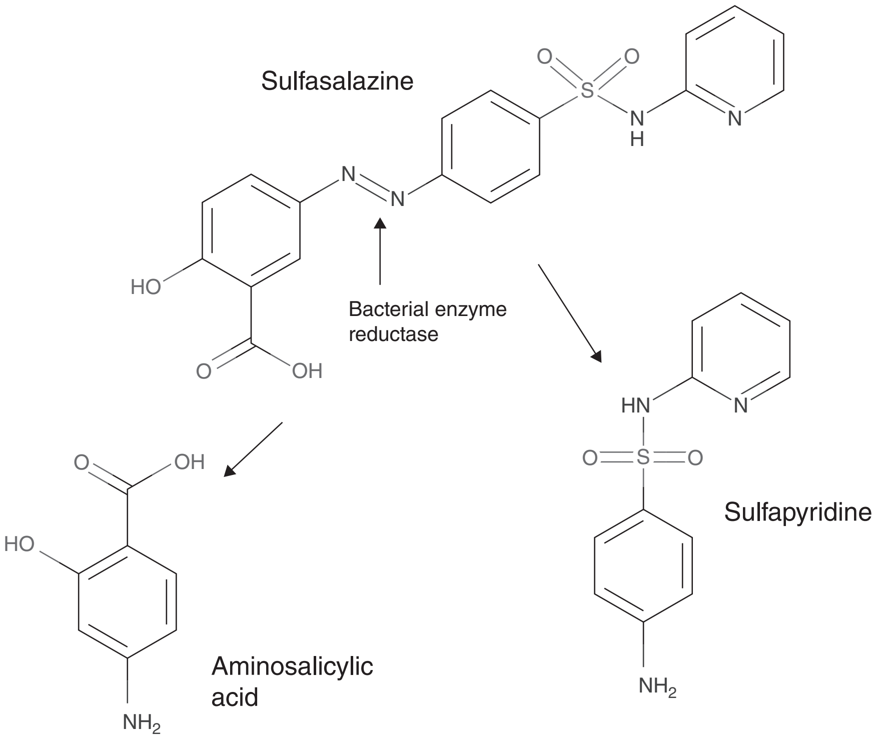


Figure 32.3 Structure of sulfasalazine, showing the reduction by bacterial enzymes in the colon (breaking the azo bond) to form 5-aminosalicylic acid and sulfapyridine. Sulfapyridine can be absorbed systemically but has no significant therapeutic effects; aminosalicylic acid has local antiinflammatory effects in the bowel.

(Figure 32.1) use alone has been reported in some studies. The pharmacokinetics are listed in Table 32.3. Sulfadiazine has been used to control plaque and gingivitis in dogs (Howell et al., 1989) and has attained concentrations in the cerebrospinal fluid (when administered IV) above the reported MIC values for many of the Enterobacteriaceae family (Vergin et al., 1984). In pigs, sulfadiazine administration was reported from various studies (Soli et al., 1990; Guise et al., 1986).

Sulfabromomethazine

Sulfabromomethazine is the brominated derivative of sulfamethazine and is considered a long-acting sulfonamide that is now rarely used. Sulfabromomethazine has a lower solubility than sulfamethazine, and single oral doses of the drug have been used to treat calf diphtheria and pneumonia, metritis, foot rot, and septic mastitis in cattle, with a repeated dose 48 hours later sometimes required. Use of sulfabromomethazine during the last 3 months of pregnancy should be avoided (Bevill, 1988).

Sulfaethoxypyridazine

Sulfaethoxypyridazine also is rarely used today. It is rapidly absorbed after oral administration to swine, sheep, and cattle and is extensively bound to plasma proteins. The parent compound and the N4-acetylated metabolite and another unidentified glucuronide conjugate seem to be the major urinary excretion products (Bevill, 1988). Sulfaethoxypyridazine can induce cataracts at some doses when fed to dogs and rats over a period of 27 and 118 weeks, respectively (Ribelin et al., 1967).

Sulfisoxazole

Sulfisoxazole has limited use today but has been used in the treatment of urinary tract infections in the dog and cat caused by susceptible bacteria (Bevill, 1988). The pharmacokinetics of sulfisoxazole has been studied in dogs, swine, and humans (Suber et al., 1981), as well as its delivery across the skin using iontophoresis (Inada et al., 1989). A formulation of combined trimethoprim–sulfisoxazole is available in some countries for injection of cattle.

Sulfachlorpyridazine

When used alone, sulfachlorpyridazine is administered most often to calves and pigs. Sulfachlorpyridazine powder, to be mixed into an oral solution (Vetisulid powder) or tablets (Vetisulid 2-gram tablets), has been administered to calves and pigs for treatment of enteritis at a dose of 33–49.5 mg/kg for calves and 44–77 mg/kg per day to pigs. There is also an injectable solution available (Vetisulid injection 200 mg/ml) for intravenous use in calves. Sulfachlorpyridazine has also been used in combination with trimethoprim and this combination is discussed in more detail in the section on potentiated sulfonamides.

Sulfachlorpyridazine is rapidly eliminated from the plasma following IV administration. Intramuscular injections in swine result in maximum blood concentrations within 30 minutes after injection, which are maintained for up to 3 hours (Bevill, 1988). A single 50 mg/kg IV dose of sulfachlorpyridazine demonstrated significantly different volume of distribution in cocks (0.34 l/kg) versus hens (0.36 l/kg), with the sulfonamide being more slowly excreted in hens (Lashev et al., 1995).

The pharmacokinetics of sulfachlorpyridazine after oral and intracardiac administrations has also been described in the channel catfish (*Ictalurus punctatus*), and the drug has been found to have a potential use in aquaculture (Alavi et al., 1993).

Other Sulfonamides

This chapter has discussed the major sulfonamides in use in veterinary medicine today, and briefly some minor sulfonamides even though their used has declined significantly. However, other sulfonamides exist that are not currently, or are no longer, used in the US markets. Other sulfonamides that may be of interest, either historically or because of use in other countries, include sulfadimethoxypyrimidine (Walker and Williams, 1972), sulfasomidine and sulfamethomidine (Bridges et al., 1969), sulfamethoxypyridazine (Garg and Uppal, 1997), sulfamethoxydiazine (Weijkamp et al., 1994), and sulfamethylphenazole (Austin and Kelly, 1966). The combination of sulfadimethoxine–sulfamethoxazole use in healthy and pneumonic pigs (Mengelers et al., 1995) has also been reported.

Previous editions of this textbook or the individual references listed here may be consulted for more in-depth information on the older and less commonly used sulfonamides not discussed in this chapter.

Potentiated Sulfonamides

The combination of sulfonamides with trimethoprim or ormetoprim (Figure 32.4) produces greater antibacterial activity than either drug used alone. Consequently, in humans, dogs, cats, horses, and occasionally other animals, these formulations are used more commonly than sulfonamides alone.

Sulfonamide and trimethoprim combinations have been reviewed in some depth by Bushby (1980) and van Miert (1994). An extensive review of trimethoprim–sulfonamide combinations in the horse is also available (van Duijkeren et al., 1994b). Although these reviews are several years old, the pharmacology and use in veterinary medicine has not changed substantially in 30 years.

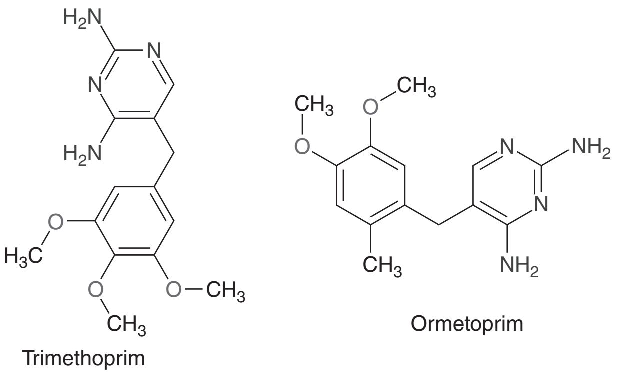


Figure 32.4 Structure of trimethoprim (left) and ormetoprim (right).

The pharmacokinetic parameters of diaminopyrimidines (trimethoprim, ormetoprim, and others) have been established for some species and are listed in Tables 32.5 and 32.6. Specific properties of these diaminopyrimidines are also discussed by Ascalone et al., 1986; Mengelers et al., 1990; Lohuis et al., 1992; Sutter et al., 1993; Wilson et al., 1987; Brown et al., 1989; Iversen et al., 1984; Vergin et al., 1984; Aschhoff, 1979.

The full range of combinations possible include a sulfonamide with trimethoprim (2,4-diamino-5-(3,4, 5-trimethoxybenzyl) pyrimidine), aditoprim (2,4-diamino-5-[4-(dimethylamino)-3,5-dimethoxybenzyl] pyrimidine), ormetoprim (2,4-diamino-5-[4,5-dimethoxy-2-methylbenzyl] pyrimidine), or tetroxoprim (2,4-diamino-5-[3,5-dimethoxy-4(2-methoxy ethoxy)benzyl] pyrimidine). These are commonly referred to as *potentiated sulfonamides.*

Trimethoprim–Sulfadiazine

Sulfadiazine is most often administered in combination with trimethoprim (e.g., Tribrissen and other veterinary products). This combination has been used for respiratory infections, urinary tract infections, urogenital infections, protozoal infections, bone and joint infections, and skin and soft-tissue infections. It is available as an oral paste (400 mg per gram, e.g., Tribrissen®) and oral suspension (Equilsul-SDZ®, 400 mg/ml). It also is available as a powder (e.g., Tucoprim®, Uniprim™) to be added to feed for horses. Feeding does not affect absorption of sulfadiazine in horses, but it may delay the absorption of the trimethoprim component. Tablets of sulfadiazine and trimethoprim (5:1 ratio) have been available in a range of sizes for small animals, but the availability of the small animal products has diminished in recent years. Injectable formulations have become less available, but

24% and 48% injections (e.g., Tribrissen®) have been used in a range of species.

Trimethoprim–sulfadiazine combination in a 1:5 ratio has been among the most popular antimicrobials in dogs, cats, and horses as a general "first-line" antimicrobial that can be useful for treating a wide variety of pathogens, in particular *Staphylococcus* spp., *Streptococcus* spp., and some gram-negative organisms, such as *Proteus mirabilis* spp., *Pasteurella* spp., and *Klebsiella* spp. However, resistance among the gram-negative bacilli *Enterobacteriaceae* can be common.

Dosing Recommendations

It is difficult to correlate plasma drug levels (and plasma elimination rates) with clinical efficacy and dosing intervals because trimethoprim is excreted faster (shorter half-life) than the sulfadiazine component; however, trimethoprim persists longer in some tissues than in the plasma and it is possible that tissue are maintained higher than plasma drug concentrations. Among the various species, doses range from 15 mg/kg twice daily, to 30 mg/kg twice daily. (See specific species recommendations.) Doses are generally listed as the combined product; that is, 30 mg/kg is equivalent to 5 mg/kg trimethoprim + 25 mg/kg of the sulfonamide.

The safety is generally acceptable for use in dogs, except for general concerns discussed for sulfonamides in this chapter. Specific toxicological studies have been conducted in both dogs and cats (Craig and White, 1976). In the Craig and White study, dogs were administered up to 300 mg/kg/day orally (10 times the recommended dose) of trimethoprim–sulfadiazine for as long as 20 days with no abnormal clinical signs or blood or serum chemistry abnormalities reported. Cats were more sensitive to adverse effects. When cats were administered 30 to 300 mg/kg/day orally for 10 to 30 days, the high dose (300 mg/kg) produced signs of lethargy, anorexia, anemia, leukopenia, and altered blood urea nitrogen (BUN).

Equine Use

Trimethoprim–sulfadiazine use in horses is common. Pharmacokinetics have been reported for horses (Tables 32.3 and 32.5) and dosing protocols have been established (McClure et al., 2015; White and Prior, 1982; Divers et al., 1981; Bertone et al., 1988). A common use in horses includes treatment of *Streptococcus equi* subsp. *zooepidemicus* and *Streptococcus equi* subsp. *equi* from horses (McCandlish and Thompson, 1979; McClure et al., 2015; van Duijkeren et al., 1994c). However, in tissue cages placed in horses, trimethoprim–sulfadiazine did not eliminate infection of *Streptococcus zooepidemicus* (Ensink et al., 2005). The inability to eliminate the infection in an infected environment may be caused by inhibitors – such as PABA and thymidine – present in abscessed and infected tissues that may inhibit the effects of these drugs.

For horses, the proper dosing regimen was reviewed by van Duijkeren et al. (1994c) and drug concentrations reported by Winther et al. (2011), and McClure et al. (2015). Drug concentrations of the oral paste are not as high as intragastric administration or the oral liquid suspension, but high enough for treatment. The conclusion reached from most studies in horses is that twice daily administration is needed because of the rapid elimination in horses – particularly of the trimethoprim component – and the need to maintain concentrations above the MIC throughout most of the dosing interval. This was confirmed in the studies by Winther et al. (2011) and McClure et al. (2015) for respiratory infections. In horses with joint infections the optimum dosage is 30 mg/kg q 12 h (Bertone et al., 1988). van Duijkeren et al. (2002) also concluded from an analysis of plasma concentrations that twice-daily administration to horses at 30 mg/kg (25 sulfadiazine; 5 trimethoprim) was necessary.

Small Animal Use

Trimethoprim–sulfadiazine combination was effective for treating urinary tract infections caused by *Staphylococcus intermedius* (now referred to as *S. pseudintermedius*) (Turnwald et al., 1986) as well as the more common pathogens such as *E. coli, Proteus mirabilis, Klebsiella pneumoniae,* and *Streptococcus* spp. (Ling and Ruby, 1979; Ling et al., 1984). Beagle dogs treated with trimethoprim–sulfadiazine (240 mg total of a 1:5 combination) once a day or the same daily dose divided into twice daily had high concentrations of both sulfadiazine and trimethoprim in their urine that greatly exceeded the MIC values for most susceptible pathogens (Sigel et al., 1981). The effectiveness for urinary tractions caused by *Enterococcus* spp. is doubtful based on the analysis by Wisell et al. (2008).

An overall success rate of 85% was reported in dogs and cats treated with a trimethoprim–sulfadiazine combination for microbial diseases involving the alimentary, respiratory, urogenital, skin, and other systems (Craig, 1972). A common use of trimethoprim–sulfadiazine is for the treatment of bacterial skin infections. Success rates of 90% (skin diseases either cured or improved) in bacterial skin infections; foot infections; interdigital abscesses; anal abscesses; and infections of the eye, ear, and mouth in dogs have been reported. A similar success rate was reported in cats (89%) with infections caused from bite wounds and other infections. Although good activity is expected against wild-type strains of *Staphylococcus pseudintermedius* isolated from dogs, methicillin-resistant strains are usually resistant.

Pharmacokinetic studies in dogs have reported that 30 mg/kg (25 sulfadiazine + 5 trimethoprim) administered once daily, should be adequate for most infections caused by susceptible organisms (Sigel et al., 1981). In a specific study in which response for treating skin infections was examined (Messinger and Beale, 1993), there was no difference in response between once- or twice-daily administration. However, the authors acknowledged that the sample size may have been too small to detect a significant difference. Supporting a once daily schedule, a study in dogs with skin infections showed that 30 mg/kg once daily is adequate (Pohlenz-Zertuche et al., 1992). These authors reported that 30 mg/kg of trimethoprim–sulfadiazine orally at 12- or 24-hour intervals were found to attain therapeutically useful concentrations of both trimethoprim and sulfadiazine in the skin (Pohlenz-Zertuche et al., 1992).

In dogs, it also has been used for *Bordetella bronchiseptica* (Powers et al., 1980), ocular infections (Sigel et al., 1981), and prostate infections. The distribution into the prostate is discussed in Section Distribution. The use for treatment of protozoan infections is discussed in more detail in Chapter 42 of this book.

Cattle Use

Trimethoprim–sulfadiazine has been used to treat infections in cattle (Slaughter, 1972), but because there are no longer any approved formulations of trimethoprim–sulfadiazine for cattle, the use has diminished. Nevertheless, the pharmacokinetics have been described in calves with values reported for tissue fluids (Shoaf et al., 1986). The pharmacokinetics of sulfadiazine and trimethoprim are reported for calves and in cattle in Tables 32.3 and Table 32.5. Calves given sulfadiazine subcutaneously (30 mg/kg) had a rapid absorption of the drug; age and diet had no effect on sulfadiazine or trimethoprim disposition in those calves (Shoaf et al., 1987). In another study by Guard et al. (1986), calves 1 day of age showed higher serum and synovial fluid concentrations of trimethoprim and sulfadiazine than did calves of 1 week or 6 weeks of age. Trimethoprim–sulfadiazine concentrations also have been documented in the cerebrospinal fluid of neonatal calves (Shoaf et al., 1989). Although rarely administered orally to cattle, as indicated in Section Oral Absorption, trimethoprim is absorbed in preruminant calves, but not absorbed in mature ruminants after oral administration (Shoaf et al., 1987).

Pig Use

Trimethoprim (8 mg/kg) –sulfadiazine (40 mg/kg) was administered orally to pigs to determine bioavailability and other pharmacokinetic parameters (Tables 32.3 and Table 32.5). Bioavailability of sulfadiazine was 89% and 85% in fasted and fed pigs, respectively, while the trimethoprim resulted in bioavailability values of 90% and 92%. After IV administration of trimethoprim (4 mg/kg) –sulfadiazine (20 mg/kg), sulfadiazine was detectable in plasma up to 30 hours after administration, while the trimethoprim was found in the plasma only during the first 12 hours after dosing (Nielsen and Gyrd-Hansen, 1994). Other species in which trimethoprim–sulfadiazine was investigated include carp (Nouws et al., 1993) and ewes (Youssef et al., 1981).

Trimethoprim–Sulfamethoxazole

The most common human formulation is trimethoprim–sulfamethoxazole (Bactrim and Septra) and it is sometimes referred to as *cotrimoxazole*. Although there are no registered veterinary formulations, the generic human formulation is inexpensive and has been used commonly in dogs and horses for oral administration.

Trimethoprim–sulfamethoxazole human formulations are available as liquid suspensions (48 mg/ml) or oral tablets (480 or 960 mg). The injectable formulation is 96 mg/ml (80 mg sulfamethoxazole + 16 mg trimethoprim). The injectable form should be given slowly IV. It has been used at a dose of 43.5 mg/kg (combined drugs) to treat CNS infections in foals.

Because of the availability of the human generic formulation of trimethoprim–sulfamethoxazole, it is often used in horses, dogs, and other species for oral administration. Many veterinarians consider trimethoprim–sulfamethoxazole to be interchangeable with trimethoprim–sulfadiazine. Although there are no side-by-side comparisons in clinical studies, there is no reason to doubt this assumption. For susceptibility testing, trimethoprim–sulfamethoxazole can be used as a surrogate to test susceptibility of trimethoprim–sulfadiazine. The pharmacokinetics have been examined in horses (Brown et al., 1988; Peck et al., 2002) (Table 32.4) and the pharmacokinetics are favorable for clinical use and dosage regimens that essentially mirror that of the use of trimethoprim–sulfadiazine.

Trimethoprim–Sulfachlorpyridazine

Even though this product is rarely used, the studies of trimethoprim–sulfachlorpyridazine have been primarily in horses. Horses given 5 mg/kg trimethoprim and 25 mg/kg sulfachlorpyridazine IV revealed an elimination half-life of 2.57 hours (trimethoprim) and 3.78 hours (sulfachlorpyridazine) and a volume of distribution of 1.51 l/kg (trimethoprim) and 0.26 l/kg (sulfachlorpyridazine) (van Duijkeren et al., 1995). Bioavailability of the same dose of sulfachlorpyridazine in a powder formulation administered in the feed was about 46%. The dose of trimethoprim–sulfachlorpyridazine of 30 mg/kg (combined drug) twice daily is recommended for most indications in horses (Van Duijkeren et al., 1995).

Sulfadimethoxine–Ormetoprim

Sulfadimethoxine has been formulated with ormetoprim to enhance the spectrum of antimicrobial activity in a similar manner to trimethoprim's enhancement of the activity of other sulfonamides. Commercial preparations of ormetoprim–sulfadimethoxine are available for dogs (Primor tablets), poultry (Rofenaid premix for feed), and for fish (Romet). These combinations offer the same advantages as trimethoprim–sulfonamide combinations because the ormetoprim component produces the same synergistic effect as trimethoprim. Pharmacokinetic parameters of ormetoprim are listed in Table 32.6.

Sulfadimethoxine–ormetoprim is available as a premix for medicated feed (Rofenaid). In this form, sulfadimethoxine–ormetoprim is used for prevention of coccidiosis in chickens and turkeys caused by susceptible *Eimeria* species and for prevention of fowl cholera. A similar formulation (Romet) also is available for treating furunculosis in salmon and trout. Sulfadimethoxine–ormetoprim oral tablets for dogs (Primor) have been used for skin and soft tissue infections, urinary tract infections, and intestinal coccidia infections. It has been administered once daily for these indications.

There are no commercially available cattle formulations of sulfadimethoxine–ormetoprim in the US However, this combination has been shown to be highly effective in treating calves with experimentally induced *Mannheimia hemolytica* pneumonia. Wilson et al. (1987) investigated the potential efficacy of a sulfadimethoxine–ormetoprim combination administered orally and IV to treat *Moraxella bovis* infections in cattle. In cattle, sulfadimethoxine–ormetoprim administered IV was effective in maintaining sufficiently high concentrations of both drugs in the tears to exceed the known MICs of 13 *Moraxella bovis* isolates and in maintaining those concentrations for approximately 6 hours. However, when the same concentration of sulfadimethoxine–ormetoprim was administered orally, sulfadimethoxine appeared in low concentrations and ormetoprim in very low or trace concentrations in the tears, indicating this combination of drugs when administered orally is not suitable for treating *Moraxella bovis* infections in cattle.

In horses, pharmacokinetics were described by Brown et al. (1989). That study administered sulfadimethoxine–ormetoprim (45.8 mg/kg:9.2 mg/kg) orally, followed by lower oral doses (22.9 mg/kg:4.6 mg/kg) at 24-hour intervals, to healthy adult mares. Sulfadimethoxine produced peak plasma concentrations 8 hours after the initial dose, and plasma concentrations above 50 μg/ml were maintained for the entire dosing schedule. Significant concentrations were also found in the synovial fluid, peritoneal fluid, endometrium, and urine, with a small amount (2.1 mg/ml) appearing in the cerebrospinal fluid approximately 100 hours after the initial dose.

The pharmacokinetic parameters of sulfadimethoxine–ormetoprim were determined in 1- to 3-day-old foals given a sulfadimethoxine–ormetoprim dose (17.5 mg/kg:3.5 mg/kg) orally (Brown et al., 1993). In the foals, sulfadimethoxine concentrations peaked at 8 hours (55 μg/ml) after the oral dose and declined to 37.6 μg/ml 24 hours after the dose.

Residues in Food Animals

Tissue residues from sulfonamide use in food-producing animals are a concern of both US government agencies and the end consumers (Bevill, 1989). FARAD (Food Animal Residue Avoidance Databank), a computerized databank of scientific and regulatory data, has specific information on the withdrawal times for sulfonamides used extralabel (www.farad.org) in food-producing animals (Riviere et al., 1986). Chapter 61 of this book address the issues of residues in food-producing animals. Detection methods for sulfonamides and metabolites have also been described (Sharma et al., 1976; Agarwal, 1992).

Sulfonamide residues were a problem in the United States for at least 30 years, having produced more drug-residue violations than any other drug, with the highest incidence occurring in pork, followed by veal and poultry. Residues in animal tissues consumed by humans are considered to be potential health hazards to humans. Toxic or allergic reactions to the sulfonamide class of antimicrobials have been reported in humans receiving therapeutic doses of sulfonamides. However, we are aware of no reports in the open literature about toxicity or other adverse reactions in humans consuming animal products containing trace amounts of sulfonamides or its metabolites. Evidence indicating that sulfonamides (in particular, sulfamethazine) may be carcinogenic in humans consuming small amounts over long periods of time (based on in vivo rat and mouse data) heightened the Food Safety Inspection Service's (FSIS) concern for controlling sulfonamide residues in food animals (USDA, 1988).

The highest rate of sulfonamide–residue violations has historically occurred in swine. Sulfamethazine and sulfathiazole are the two most commonly used sulfonamides in swine feeds today. However, sulfamethazine was responsible for most of the sulfonamide–residue violations (97%) because it had been commonly added to swine feeds and its longer half-life when compared to that of sulfathiazole (12.7 versus 1.2 hours). The primary reasons for the occurrence of violative levels of sulfonamides in pork were failure to observe drug withdrawal time, improper feed mixing, and improper cleaning of feed-mixing equipment, causing a cross-contamination of feed (Bevill, 1984, 1989). During the late 1970s, 13% of swine livers were found to be in violation of federal sulfonamide tissue concentrations. At that time the maximum amount of sulfonamide (parent compound) permitted in animal tissues was 0.1 ppm, with a 7-day withdrawal period. Drug manufacturers at this time increased the withdrawal time for sulfonamides used in animal feed from 7 to 15 days, and, by 1980, the violation rate in liver tissue had fallen to 4%. In 1987, the rate was reported to be 3.8% (Augsburg, 1989), with the rate decreasing significantly by the end of the 1990s. Part of the persistence of even these low levels may also be related to the ease of cross contamination seen after water medication (Mason et al., 2008).

In veal calves presented for slaughter, similar problems with sulfamethazine residues have been reported. The prevalence rate of sulfamethazine violations in veal calves was 1.9% in 1979 and 2.9% in 1981. Reasons for violations in this species include administering the drug to calves by individuals unaware of the drug withdrawal time constraints, unknowingly selling calves treated with sulfonamides, not following drug label directions, not seeking professional advice regarding drug use, and failing to maintain drug use records (Bevill, 1989).

Legal control of veterinary drugs is discussed in Chapter 55 of this book, and more information about residues in food-producing animals is presented in Chapter 61 of this textbook and also in previous editions. Several references are also available on this subject (Kaneene and Miller, 1992; Bevill, 1984; Dalvi, 1988; Rosenberg, 1985).

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33

β-Lactam Antibiotics: Penicillins, Cephalosporins, and Related Drugs

Mark G. Papich

In 1928, Alexander Fleming observed that a *Penicillium* mold contaminating a Petri dish culture of staphylococci colonies was surrounded by a clear zone free of growth. Fleming cultured the contaminating mold on a special medium and demonstrated that the culture broth contained a potent antibacterial substance that was relatively nontoxic to animals, but was active against a variety of gram-positive organisms. He named the substance *penicillin*. In 1940, penicillin was isolated in the form of a brown, impure powder and was the most powerful chemotherapeutic agent known at that time. Since then, more than 40 penicillins have been identified. Some occur naturally; others are biosynthesized (semisynthetic penicillins).

In 1945, *Cephalosporium acremonium* was isolated from raw sewage. The first cephalosporin, cephalosporin C, was derived from this fungus. All other cephalosporins are semisynthetic antibiotic derivatives of cephalosporin C. The first cephalosporin was available for clinical use in 1964. Since those first cephalosporins, they have developed through first, second, third, and fourth generations, all of which are used in veterinary medicine. There is now a commercially available *fifth* generation used in humans. The reader may notice differences in spelling. The older cephalosporins, derived from the fungus are spelled *ceph*, whereas the newer semisynthetic derivatives are spelled *cef*.

Although penicillins, penicillin derivatives, and cephalosporins are still the most commonly used β-lactam antibiotics, much progress has been made in the development of new β-lactams during recent years. Most notably, these include the β-lactamase inhibitors (e.g., clavulanic acid), new cephalosporins, the carbapenems, also known as *penems* (e.g., imipenem, meropenem, ertapenem, doripenem), and the monobactams (e.g., aztreonam) (Abraham, 1987).

Mechanism of Action of β-Lactam Antibiotics

β-lactam antibiotics exert their effects by preventing bacterial cell wall synthesis and disrupting bacterial cell wall integrity. These drugs are considered *bactericidal* in a time-dependent manner. They kill bacteria by inhibiting or weakening the cell wall. The cell wall of bacteria consists of alternating units of *N-acetylglucosamine* and *N-acetylmuramic acid,* which are cross-linked by short strands of peptides. A transpeptidation reaction is responsible for cross-linking the strands to form a strong, netlike structure. Inhibition of this transpeptidation reaction by acetylating the enzyme is one of the sites of action for β-lactam antibiotics. Interference with transpeptidation results in a weak cell wall and rupture of the bacteria.

The binding sites for β-lactam antibiotics are called penicillin-binding proteins (PBPs), which are the enzymes that form the cell wall. There can be anywhere from two to eight distinct PBPs in bacteria, which are numbered according to their molecular weight. The most common PBPs affected by β-lactams are PBP-1, -2, or -3. Some of the variation in spectrum of action and bactericidal action of β-lactam antibiotics can be related to their relative affinity for different PBPs. For example, inhibition of PBP-1a and -1b generally cause lysis, PBP-2 results in rounded cells called *spheroblasts,* and PBP-3 produces long filamentous forms. The drugs that cause rapid lysis (for example, carbapenems) are the most bactericidal and have highest affinity for PBP-1.

On the other hand, mutations in the enzyme, produced by the resistance gene *mecA*, produces PBP-2a, a penicillin-binding protein that resists binding of the β-lactam drugs and renders bacteria with this gene resistant. The PBP-2a target is the basis for resistance to

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methicillin and oxacillin, known as *methicillin-resistant staphylococci (MRS)* (Weese, 2005).

In order to reach the site of action, these drugs first must penetrate the outer layer of the bacteria. They penetrate the outer layer of bacteria through a pore (a porin protein) that is ordinarily present in bacteria to allow nutrients to enter the cell. It is generally easier to reach the target site in gram-positive bacteria and they usually are more susceptible to the effects of β-lactam antibiotics than gram-negative bacteria. Gram-negative bacteria may have a thick outer membrane and have pores more difficult to penetrate. The drugs most effective against gram-negative bacteria are those that can rapidly penetrate the outer membrane of the cell wall.

Pharmacokinetic–Pharmacodynamic (PK-PD) Properties

The β-lactam antibiotics are time dependent in their activity (Turnidge, 1998). β-lactam antibiotics are slowly bactericidal because of the slow rate of acetylation of the PBP, and the time of drug concentration above the minimum inhibitory concentration (MIC) (T > MIC) is important to clinical success (Drusano, 2004). At low exposure of β-lactams, not all of the available PBP are acetylated and bacterial stasis occurs (bacteriostatic effect). As the maximum number of PBP become acetylated, bactericidal activity occurs. Once maximum acetylation of the enzymes occurs the killing rate does not increase further, which explains the time-dependent effect of β-lactams, rather than a concentration-dependent effect. In some cases, drug concentrations can fall below the MIC for treating staphylococcal infections and still attain a cure because of a postantibiotic effect (PAE), but a PAE does not occur against gram-negative bacilli (Zhanel et al., 1991). Additionally, since the MICs are lower for gram-positive bacteria, longer dose intervals may be possible for infections caused by gram-positive as compared to gram-negative bacteria because it is easier to keep the plasma concentration above the MIC.

The optimum duration of plasma concentrations above the MIC has varied among studies, but a general assumption is that the drug concentration should be above the MIC for 50% – and perhaps less – of the dosing interval (Turnidge, 1998; Drusano, 2004). This may vary depending on the immune competence of the animal and specific drug class. The carbapenem group of drugs (for example, imipenem and meropenem), are used with increasing popularity in small animal practice. These drugs are more bactericidal than penicillins and cephalosporins and the T > MIC for successful therapy may be less for these drugs than other β-lactams (for example, 30% of the dose interval). When immune competence is a question – for any of the β-lactams – increasing the time above MIC is advised.

Dosage regimens for the β-lactam antibiotics should consider these pharmacodynamic relationships. Therefore, for treating a gram-negative infection, especially a serious one, it is necessary to administer many of the penicillin derivatives and cephalosporins three to four times per day if they have short half-lives. Some of the third-generation cephalosporins have long half-lives and less frequent regimens have been used for some of these drugs (e.g., ceftiofur–Naxcel, cefovecin–Convenia, and cefpodoxime proxetil–Simplicef). For highly susceptible gram-positive infections, less frequent administration may be acceptable. Note that the indications on the FDA-approved label for veterinary β-lactams may be aimed at these highly susceptible gram-positive pathogens and may not be appropriate for gram-negative bacteria with higher MIC values. Specific examples to illustrate this point are provided in this chapter.

Microbial Resistance to β-Lactam Antibiotics

Three independent factors determine the bacterial susceptibility to β-lactam antibiotics: (i) production of β-lactamases, (ii) decreased penetration through the outer cell membrane to access the cell wall enzymes (which includes efflux pump mechanisms), and (iii) the resistance of the target (PBP) to binding by β-lactam agents (Gold and Moellering, 1996; Frere et al., 1991). An example of an altered target (PBP) is the one found in methicillin-resistant isolates, mentioned in Section Mechanism Of Action Of β-Lactam Antibiotics.

β-Lactamase Enzymes

The elaboration of β-lactamases, enzymes that inactivate the drugs by hydrolyzing the β-lactam ring, is the major mechanism of drug resistance (Jacoby and Munoz-Price, 2005). Bacteria produce β-lactamases that possess a range of physical, chemical, and functional properties. Some β-lactamases are specific for penicillins (penicillinases), some are specific for cephalosporins (cephalosporinases), and others have affinity for both groups.

The genes that code for β-lactamases can occur via mutations in chromosomes, or they may be transferred by genetic elements. The genetic elements carrying these genes – which may be transferred among bacteria – include plasmids, which may be organized into integrons or carried on tranposons. A transposon may move (transpose) from DNA to plasmids, and vice versa. Integrons may be part of a transposon. The integrons may also contain genes that code for resistance to other drugs – thus producing multidrug resistance.

There are many β-lactamase enzymes that are capable of hydrolyzing the cyclic amide bond of the β-lactam structure and inactivating the drug. Investigators have described over 190 unique β-lactamase proteins that have this ability. Classification schemes may vary, and β-lactamase enzymes have been categorized according to molecular structure and substrate, bacterial type (gram-negative versus gram-positive), transmission (plasmid coded versus chromosomal coded), and whether they are inducible or constitutive. The Ambler Class (Class A, B, C, and D), which uses amino acid sequencing, is now widely accepted (Rice and Bonomo, 2000; Jacoby and Munoz-Price, 2005). Class A enzymes are the most important in veterinary medicine. These include those listed in the following sections.

Staphylococcal β-Lactamase

These are produced by coagulase-positive *Staphylococcus* sp. and some coagulase-negative strains. The synthesis of these enzymes is determined by specific resistance genes and the enzymes are exocelluar. These enzymes typically do not inactivate cephalosporins and antistaphylococcal penicillins (e.g., isoxazolyl penicillins such as oxacillin or dicloxacillin). These β-lactamases can be inactivated by β-lactamase inhibitors (e.g., clavulanate acid, tazobactam, and sulbactam).

Gram-Negative β-Lactamases

This is a very diverse group that can arise through mutation or via transferable genetic elements. These enzymes are more wide spectrum and can hydrolyze penicillins (penicillinases), cephalosporins (cephalosporinases), or both. β-lactamases produced by Enterobacteriaceae such as *E. coli* belong to the TEM, SHV, and CTX-M, families, as well as other less common groups. Some, but not all, of these enzymes are inhibited by β-lactamase inhibitors (e.g., clavulanate, sulbactam). Gram-negative bacteria secrete small amounts of β-lactamases into their periplasmic space, allowing for optimal location of the enzyme to degrade the β-lactam upon entry into the organism. The advantage of newer drugs such as third-generation cephalosporins (also called *oxyimino-β-lactams*) and carbapenems is their ability to remain stable to gram-negative β-lactamases.

Among the most serious of the gram-negative β-lactamases are the ESBLs – extended-spectrum β-lactamases. These β-lactamases will cause resistance to even the most active (extended-spectrum) cephalosporins. These enzymes are generally acquired through horizontal gene transfer (plasmids or transposons), but resistant clones also can spread in the local environment or hospital. Regardless of the mechanism, it presents a challenge to clinicians. Bacteria positive for ESBL can be difficult to treat because of limited drug options, and many are resistant to other classes of antibiotics. A more recently emerging β-lactamase resistance is the production of carbapenemase. This enzyme will inactivate the valuable carbepenem class of drugs. These bacteria have been identified as *carbapenem-resistant Enterobacteriaceae* (CRE), and include the *Klebsiella pneumoniae* carbapenemase (KPC). Bacteria that carry this resistance are very difficult to treat because there are so few available treatment options. Fortunately, this mode of resistance has not yet become a clinical problem in veterinary medicine, but there are concerns about its occurrence in animals (Abraham et al., 2014).

Resistance Caused by Reduced Access to Binding Sites

Gram-negative bacteria can produce a cell wall with a modified outer membrane that is no longer permeable to β-lactam antibiotics. These impenetrable porin proteins can resist entry by β-lactam drugs. Reducing the permeability of the outer membrane limits antibiotic entry into the bacteria by down-regulation of the porin protein, or by replacement of the porin with a channel that resists entry by the drug. The porins can also delay or slow the rate of entry, thus making the drugs more susceptible to attack by β-lactamase enzymes. Therefore, this mechanism can enhance resistance produced by the elaboration of β-lactamases.

Another mechanism that inhibits access to target sites is the efflux of β-lactams out of the bacteria. This mechanism actively transports the antibiotic out of the cell. When these pumps are expressed, it can produce a high level of resistance and can affect multiple drug classes – thus conferring multidrug resistance (MDR). The MDR efflux pumps may be carried on chromosomes or transferred by plasmids.

Penicillins

General Pharmacology

Penicillin G is historically important because it was the first antibiotic introduced in medicine. Alexander Fleming discovered penicillin in 1928, but it was not used clinically until the early 1940s. Much of the early supply was designated for military use during World War II. It remains a popular antibiotic and is still the drug of choice for initial treatment of many infections in horses and cattle. It has little usefulness in small animals because of the high incidence of resistance.

Unitage

Penicillin is one of the few antibiotics that is still measured in terms of units rather than weight in mg or μg. One unit of penicillin represents the specific activity in

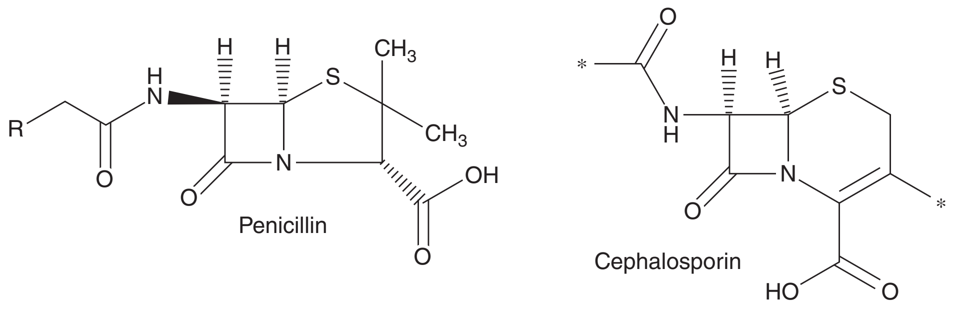

Figure 33.1 Structure of penicillins (left) and cephalosporins (right). Penicillins are composed of a five-member thiazolidine ring; cephalosporins are composed of a six-member dihydrothiazine ring. The structures can be modified into different drugs by replacing the R-groups or asterisk (∗) with other functional groups. The most critical feature of the structure to exert its antibacterial action is the β-lactam ring. If the β-lactam ring is broken (hydrolyzed), the drug is inactive.

0.6 μg of sodium penicillin based on the International Standard for Penicillin. Thus, one mg of penicillin sodium represents 1,667 units of penicillin. Doses of other β-lactams are expressed in weight (mg) rather than units.

Structure

Penicillin contains a fused ring system, the β-lactam thiazolidine (Figure 33.1). The physical and chemical properties, especially solubility, of penicillins are determined by the acyl side chain and the cations used to form salts.

Hydrolysis is the main cause of penicillin degradation, which can occur via bacterial enzymes (β-lactamases). This reaction also can occur from drug interactions (for example, in the syringe when penicillin is mixed with another drug). Some penicillins are poorly orally absorbed because they are unstable in the stomach and hydrolyzed by gastric acid. Penicillin is incompatible with heavy metal ions, oxidizing agents, and strong concentrations of alcohol.

The penicillins are listed in categories based on their synthesis and spectrum of action. These include the natural penicillins (e.g. penicillin G), aminopenicillins (e.g. ampicillin, amoxicillin), antistaphylococcal penicillins (e.g. oxacillin), and the extended-spectrum penicillins (e.g. piperacillin). These are discussed in more detail in specific sections later in the chapter.

Antimicrobial Activity

The natural penicillins are active against many *Streptococcus* spp. and nonpenicillinase-producing *Staphylococcus* spp. They are active against some gram-positive, but only selected gram-negative bacteria, which include *Arcanobacterium* (formerly *Actinomyces*), *Listeria monocytogenes,* and *Pasteurella multocida*. Therefore, these drugs are narrow-spectrum drugs that are active against non–β-lactamase–producing gram-positive bacteria but few gram-negative bacteria. Many anaerobic bacteria are susceptible, including *Fusobacterium, Peptococcus, Peptostreptococcus*, and some strains of *Bacteroides* (except *Bacteriodes fragilis* group) and *Clostridium.* These drugs are also active against most spirochetes, including *Leptospira* and *Borrelia burgdorferi*. Natural penicillins are consistently inactive against *Pseudomonas*, most Enterobacteriaceae, and penicillinase-producing *Staphylococcus* spp.

Aminopenicillins are active against the bacteria that are susceptible to penicillin G. They can penetrate through the outer membrane of gram-negative bacilli better than penicillin, which increases the spectrum to include some Enterobacteriaceae, including strains of *E. coli, Proteus mirabilis*, and *Salmonella;* however, most are resistant. Aminopenicillins are inactive against *Pseudomonas, Bacteroides fragilis*, and penicillinase-producing *Staphylococcus* spp. There are only subtle differences in antimicrobial activity between amoxicillin and ampicillin, and for susceptibility testing purposes, they are considered equivalent (CLSI, 2015).

The antistaphylocccal penicillins include methicillin and nafcillin as well as the isoxazolylpenicillins (oxacillin, cloxacillin, and dicloxacillin). The isoxazolylpenicillins are considered as a group because of their structural similarity. These semisynthetic penicillins are active against many penicillinase-producing *Staphylococcus* spp. that would ordinarily be resistant to penicillin G and the aminopenicillins. They also have some activity against other gram-positive and gram-negative bacteria and spirochetes. They are rarely, if ever, used clinically except for intramammary products that contain cloxacillin.

The extended-spectrum penicillins have the most activity against gram-negative aerobic and anaerobic bacteria of all of the penicillin groups. The drugs are active against many strains of Enterobacteriaceae and some strains of *Pseudomonas*. Carbenicillin and ticarcillin are active against some strains of *E. coli, Morganella morganii, Proteus* spp., and *Salmonella*. In addition to these organisms, mezlocillin and piperacillin are active against some strains of *Enterobacter, Citrobacter, Klebsiella*, and *Serratia*. These extended-spectrum penicillins have some activity against gram-positive aerobic and anaerobic bacteria but have no advantages against these organisms compared to penicillin G and aminopenicillins. Extended-spectrum penicillins are generally more active against *Bacteroides fragilis* than are other available penicillins. Of this group, few are available and used clinically. Ticarcillin and ticarcillin–clavulanate, once popular for injectable use, is no longer available. Carbenicillin is no longer available. Piperacillin–tazobactam is the most popular and widely available of this group.

Susceptibility Testing

The CLSI has approved breakpoints for animals for most of the penicillins and derivatives (CLSI, 2013, 2015). These breakpoints often reflect the accepted clinical use of the drug and not necessarily the approved label indication. For example, the breakpoint for procaine penicillin G was established using a dose of 22,000 U/kg IM, consistent with good clinical practice, rather than the FDA-approved label dose of 7,500 U/kg. CLSI approved breakpoints are listed in Table 33.1.

Pharmacokinetics

Ampicillin and amoxicillin have been the most studied of the penicillin group and Table 33.2 lists the pharmacokinetic parameters in several domestic species. Penicillin G pharmacokinetics are presented in Table 33.3. When sodium salts of these drugs are injected, maximum blood concentrations occur usually within an hour.

Absorption

For the penicillin G formulations, distinct absorption patterns are observed, depending on which form is administered, the formulation, and the site of injection. The long-acting formulations (procaine- or benzathine–penicillin) are given IM or SC (never IV). The slow absorption from the injection site prolongs the plasma concentration. Injections of penicillin G IM to horses produced prolonged plasma concentrations, even when the sodium salt of penicillin was administered (Uboh et al., 2000). The half-life was longer from the procaine formulation, but at 24 hours, concentrations were similar in horses, regardless of whether the procaine or potassium formulation of penicillin G was administered. Benzathine formulations produce lower, but much longer, plasma concentrations because of insolubility and extremely slow absorption from the injection site (Papich et al., 1994).

For all the penicillins, as expected, the maximum plasma concentration will be lower, and the time to reach maximum concentration delayed, from an IM route versus IV route. More rapid absorption occurs from IM injection than SC. Also, there are differences depending on the location in large animals, with an injection in the neck muscle being absorbed faster than an injection in gluteal muscle (Papich et al., 1993). This pattern of absorption has been shown for other penicillins in cattle and horses (Firth, 1986), in which injections in the neck muscle are absorbed more rapidly and completely than injections in the rear leg.

For ampicillin and amoxicillin, these can be administered IM or IV as sodium salts, or they may be formulated as the trihydrate form that is more stable in aqueous solutions and may be administered SC or IM to produce a more prolonged absorption phase and a longer duration of activity (Traver and Riviere, 1982). Sodium salts of the other penicillin derivatives are no longer commercially available for IM or IV administration (e.g., ticarcillin sodium, piperacillin sodium, etc.).

Penicillin is easily inactivated in the acidic pH of the stomach (a pH of 6–6.5 is optimum for chemical stability), and therefore is not absorbed orally (except the acid-stable penicillin V, which is discussed under the specific formulations below). Because oral absorption of penicillin G is not sufficient to be of therapeutic value in animals this route is not used.

Amoxicillin and ampicillin are often administered to small animals. Amoxicillin differs from ampicillin only by the addition of a hydroxyl group (Figure 33.2). This decreases the lipophilicity of amoxicillin, but increases the oral absorption, whereby systemic availability of amoxicillin administered orally is higher than a similar dose of ampicillin (Watson et al., 1986). For example, absorption of ampicillin in dogs and cats has been reported to range from 30 to 40%, and for amoxicillin 64 to 68% (Küng and Wanner, 1994). Ampicillin had wide variation of oral absorption in cats (18% for suspension; 42% for capsule), depending on the dosage form (Mercer et al., 1977). Amoxicillin also has twice the systemic bioavailability of ampicillin when administered orally in pigs and preruminant calves. Other penicillin derivatives may have low oral absorption, which limits their clinical usefulness. Cloxacillin is absorbed poorly in dogs (Watson et al., 1986; Dimitrova et al., 1998) and with its short half-life is unsuitable for therapy in dogs.

The effect of feeding on oral absorption of these drugs has been debated, and results have varied, depending on the conditions of the study. The most complete study on this subject was reported by Watson et al. (1986). They found that feeding dogs inhibited oral absorption of amoxicillin, ampicillin, penicillin V, and cloxacillin. The effect was modest for ampicillin and amoxicillin (feeding decreased ampicillin tablets by 38%, but only 20–25% for amoxicillin). Feeding had a large effect for cloxacillin, decreasing oral absorption by 74%.

Aminopenicillins are absorbed poorly in horses and ruminants following oral administration.

Table 33.1 Susceptibility breakpoints for penicillin and derivatives (as approved by CLSI, 2013, 2015).

Species	Body site	Antimicrobial agent	MIC interpretive criteria (µg/ml) S	I	R	Comments
Dogs	Skin, soft tissue (*E. coli*)	Ampicillin	≤ 0.25	0.5	≥ 1.0	Ampicillin may be used to test for amoxicillin. Systemic breakpoint derived from microbiological, PK-PD data. For dogs, the dose of amoxicillin was 22 mg/kg every 12 hours orally.
Dogs	Skin, soft tissue (gram-positive cocci)	Ampicillin	≤ 0.25	–	≥ 0.5	Ampicillin is used to test for susceptibility to amoxicillin and hetacillin. Systemic breakpoint derived from microbiological, PK-PD data. For dogs, the dose of amoxicillin was 22 mg/kg every 12 hours orally.
Dog	UTI	Ampicillin	≤8	–	–	A breakpoint of ≤8 can be used for uncomplicated UTI to account for high concentration in urine.
Dogs	Skin, soft tissue	Amoxicillin–clavulanate	≤0.25/ 0.12	0.5/0.25	≥1/0.5	Amoxicillin–clavulanate breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and PK-PD analysis of amoxicillin in dogs. The dosage regimen used for PK-PD analysis of amoxicillin was 11 mg/kg administered every 12 hours orally.
Dogs	UTI	Amoxicillin–clavulanate	≤8/4			A breakpoint of ≤8/4 can be used for uncomplicated UTI to account for high concentration in urine.
Dogs	Skin, soft tissue, UTI	Piperacillin–tazobactam	≤8/4	≤16/4	≤32/4	Breakpoint was derived from an examination of MIC distribution data, and PK-PD analysis of piperacillin in dogs at a dosage of 350 mg/kg every 6 hours, intravenously, or 3.2 mg/kg/hour constant rate IV infusion.
Cats	Skin, soft tissue, UTI	Amoxicillin–clavulanate	≤0.25/ 0.12	0.5/0.25	≥1/0.5	Amoxicillin–clavulanic acid breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and PK-PD analysis of amoxicillin in cats at a dosage of 12.5 mg/kg (amoxicillin) administered every 12 hours orally.
Horse	Respiratory, soft tissue	Penicillin G	≤ 0.5	1	≥ 2	Breakpoints derived from microbiological, PK data (using accepted clinical, but extralabel doses), and PD data. The dose of procaine penicillin G modeled was 22000 U/k, IM, every 24 hours.
Horse	Respiratory Disease	Ampicillin	≤ 0.25	–	–	For strains yielding results suggestive of a "nonsusceptible" category, organism identification and antimicrobial susceptibility test results should be confirmed.
Swine	Lung (SRD)	Ampicillin	≤ 0.5	1	≥ 2	Breakpoints derived from microbiological data using ampicillin, PK data from a dose of 15 mg/kg IM of amoxicillin once daily, and PD data.
Swine	Lung (SRD)	Penicillin G	≤ 0.25	0.5	≥ 1	Breakpoints derived from microbiological, pharmacokinetic data (using accepted clinical, but extralabel doses), and pharmacodynamic data. The dose of procaine penicillin G modeled was at a dose of 33,000 U/kg, IM by needle in the neck, every 24 hours.
Cattle	Lung (BRD)	Penicillin G	≤ 0.25	0.5	≥ 1.0	Breakpoints derived from microbiological, PK data (using accepted clinical, but extralabel doses), and PD data. The dose of procaine penicillin G modeled was 22000 U/k, IM, every 24 hours.
Cattle	Mastitis	Penicillin–novobiocin	≤ 1/2	2/4	≥4/ 8	Mastitis use only.

BRD, bovine respiratory disease; SRD, swine respiratory disease; PK, pharmacokinetic; PD, pharmacodynamic; R, resistant; I, intermediate; S, susceptible; UTI, urinary tract infection.

Table 33.2 Ampicillin and amoxicillin pharmacokinetics (mean values)

Species	Dose (mg/kg)	$T_{1/2}$ (hour)	Vd (l/kg)	CL (ml/kg/hour)	C_{max} (µg/ml)	T_{max} (hour)	F (%)	Reference
Ampicillin trihydrate IM administration								
Calves	11				2.62			Martinez et al., 2001
Pigs	6.6				3.25			Martinez et al., 2001
Horse	11				1.48	1		Beech et al., 1979
Horse	22				2.9	6		Beech et al., 1979
Horse	20				2.49	6		Brown et al., 1982
Cattle	17	6.66	4.49	467.8				Gehring et al., 2005
Ampicillin sodium IV								
Horses		0.62	0.18	210	6.7–9.7			Sarasola and McKellar, 1993
Horses	10	0.725	0.303	268	59.9			Sarasola and McKellar, 1992
Horses		1.55						Durr, 1976
Horses		1.41	0.17					Bowman et al., 1986
Horses		0.75	0.21					Horspool et al., 1992
Horses	15	1.72	0.705	285				Ensink et al., 1992
Horses	10	0.7	0.2628	365.4				Sarasola and McKellar, 1995
Cats		1.22						Mercer et al., 1977
Pigs		0.55						Galtier and Charpenteau, 1979
Dogs	15	1.35	0.679	387				ten Voorde et al., 1990
Sheep	10	0.78	0.156	372.6				Oukessou and Toutain, 1992
Ampicillin Sodium IM								
Cows	10	2.3			6.18	1.5		Nelis et al., 1992
Horses	15	2.3	0.71	209.8	31.1	0.32		Van Den Hoven et al., 2003
Horses	11				10	0.5		Beech et al., 1979
Horses	22	2			12.88	0.5		Traver and Riviere, 1982
Dogs	15	5.2–5.5				0.92–1.03		ten Voorde et al., 1990
Ampicillin Oral								
Dogs	14.5	0.9			4.6			Nelis et al., 1992
Dogs	10	0.96			3.9	1.6		Watson et al., 1986
Foal	20				5.0	1		Brown et al., 1984
Cat							42	Mercer et al., 1977
Horse	15				0.84	0.69	2	Ensink et al., 1996
Amoxicillin								
Foals (30 days) IM	22	0.991	0.986	691	23.21			Carter et al., 1986
Foals (6–7 days) IV	20	0.74	0.369	343.2				Baggot et al., 1988
Foals (6–7days) oral	20	1.09			6.23	2	36.2	Baggot et al., 1988
Horses IV	10	0.657	0.325	340.8				Wilson et al., 1988
Horses IM					3.9–11.9			Evans et al., 1971
Horses oral	20	0.85			11.05	0.3	10.4	Wilson et al., 1988
Horses oral	20	0.75			2.03		5	Ensink et al., 1992
Horses IV	10	1.43	0.556	273				Ensink et al., 1992
Dogs IV	20	1.3	0.312	204				Küng and Wanner, 1994
Dogs IV	15	1.18	0.449	270				ten Voorde et al., 1990
Dogs IM	15	6.98–9.02			7.64–8.13	1.61–1.89		ten Voorde et al., 1990
Pigs IV	8.6	1.8	0.55, 0.63	370, 520				Agersø and Friis, 1998
Pigs IM	14.7	15.5			5.1	2	83	Agersø and Friis, 1998
Sheep IV	10	0.77	0.22	606				Craigmill et al., 1992
Sheep IV	20	1.43	0.18	90				Carceles et al., 1995
Goats IV	10	1.15	0.47	684.6				Craigmill et al., 1992
Goats IV	20	1.13	0.18	110				Carceles et al., 1995
Dogs oral		1.06	0.284	182				Marier et al., 2001
Dogs oral	16.9	1.52	0.71	460	11.4	1.38		Vree et al., 2003
Dogs oral	21	1.5			18–21	1.4–2	64–77	Küng and Wanner, 1994
Dogs oral	10	1.4		8.1	1.6			Watson et al., 1986
Pigs oral	10	9, 9.9	2.25		0.8, 1.6	1.9, 3.6	28, 33	Agersø et al., 1998
Pigs oral	20	4.2			7.5	1.5		Jensen et al., 2004

Vd, volume of distribution; CL, systemic clearance; $T_{1/2}$, half-life; C_{max}, peak concentration; T_{max}, time of peak concentration after absorption; F, fraction of dose absorbed.

Table 33.3 Pharmacokinetic parameters for penicillin in animals

Species	Form	Dose (Units/kg)	Route/Site	C_{max} (μg/ml)	T_{max} (hours)	$T_{1/2}$ (hours)	Reference
Calves (6–9 months)	Potassium	10,000	IM/Neck	4.71 ± 3.86	1 to 1.5	–	Bengtsson et al., 1989
Calves (6–9 months)	Procaine	30,000	IM/Neck	1.55 ± 0.33	1.5 to 6	–	Bengtsson et al., 1989
Cattle	Procaine	66,000	IM/Neck	4.24 ± 1.08	6.00 ± 0.00	8.9	Papich et al., 1993
Cattle	Procaine	66,000	SC/Neck	1.85 ± 0.27	5.33 ± 0.67	17	Papich et al., 1993
Cattle After 5-day administration	Procaine	24,000	IM/Gluteal	0.99 ± 0.04	5.33 ± 0.67	17	Papich et al., 1993
Cattle	Procaine	66,000	IM/Gluteal	2.63 ± 0.27	6.00 ± 0.00	17	Papich et al., 1993
Cattle	Procaine					9.37 ± 3.4	Craigmill et al., 2004[a]
Horses	Sodium	10,000	IV/Jugular				Love et al., 1983
Horses		20,000	IV/Jugular				
Horses		40,000	IV/Jugular				
Horses	Procaine	10,000	IM/Gluteal				Sullins et al. 1984
Horses		20,000	IM/Gluteal				
Horses		40,000	IM/Gluteal				
Horses	Procaine	22,000	IM/Gluteal	1.42 ± 0.22	3		Stover et al., 1981
Horses	Procaine	22,000	IM/neck	1.8	3.5	24.7	Uboh et al., 2000
Horses	Potassium	22,000	IM/neck	5.8	1	12.9	Uboh et al., 2000
Horses	Procaine	20,000	IM	1.57 ± 0.44	4.77 ± 0.26	16.4 ± 8.0	Mean of 3 studies[b]
Foals (0–7 day)	Procaine	22,000	IM Semimembranous	2.17 ± 0.27	2		Brown et al., 1984
Pigs	Procaine	15,000	IM neck	1.47	1.20	7.78	Papich (unpublished)
Pigs	Procaine	66,000	IM neck	5.40	1.0	14.13	Papich (unpublished)

IM, intramuscular; IV, intravenous; SC, subcutaneous; C_{MAX}, peak concentration; T_{MAX}, time of peak concentration after absorption.

[a] Craigmill et al. (2004). Craigmill's analysis used 18 published papers, 28 data sets, and 288 data points. They also reported a volume of distribution/F of 13 l/kg (± 7.46).

[b] Mean values (± standard deviation) is available for more than one study.

They can be absorbed in preruminant calves, but not ruminating (6 weeks of age) calves (Soback et al., 1987a). Oral absorption of ampicillin in adult horses has been reported to be only 2–3.5% (Ensink et al., 1996; Sarasola and McKellar, 1994). Systemic availability of oral amoxicillin is higher than ampicillin in adult horses but is only 2–10% (Wilson et al., 1988; Ensink et al., 1992, 1996; Baggot, 1988; Sarasola and McKellar, 1994); still too low to be practical for dosing. In foals, oral absorption of amoxicillin has been higher at 36.2–42.7% (Baggot et al., 1988), but this is a seldom-used route of administration.

Elimination

The elimination half-life for IV penicillin is short (0.5 to 1.2 hours). Slow-release formulations are designed to lengthen this half-life. For example, because of slow release from the injection site, procaine penicillin may produce a terminal half-life of 20 hours or more and can maintain concentrations against susceptible bacteria for 24 hours after a single injection. The prolonged terminal half-life is caused by slow absorption (see the Section Absorption), rather than slow elimination, which is referred to as the "flip-flop effect" determining the plasma profile. All penicillins rely on renal elimination (primarily tubular secretion) and reach high drug concentrations in the urine.

Distribution

Penicillins diffuse into extracellular fluid easily unless protein binding is high. Protein binding is low to moderate in most species, ranging from approximately 30 to

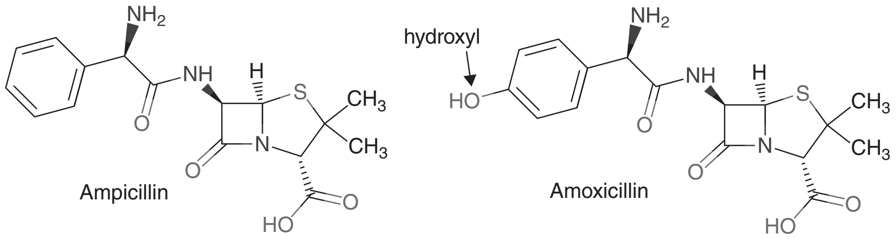

Figure 33.2 Structure of ampicillin (left) and amoxicillin (right).

60%. Penicillin is a weak acid with a pK_a of 2.7, therefore it is mostly ionized in the plasma and the volume of distribution is moderate. For example, volume of distribution (Vd) listed in some studies is 0.2 to 0.3 l/kg. In some studies, and in some species, however, it may be as high as 0.6 to 0.7 l/kg. These values represent a distribution to extracellular fluid, and possibly reaching moderate intracellular concentrations. Sufficient concentrations are attained for susceptible bacteria in kidneys, synovial fluid, liver, lung, skin, and soft tissues (Stover et al., 1981; Brown et al., 1982; Beech et al., 1979). Penicillins do not penetrate the blood–brain barrier to reach concentrations in the central nervous system (CNS) to a large extent, but they have been used to treat infections of the CNS when administered at high doses.

Metabolism

Penicillin G, penicillin V, nafcillin, ticarcillin, and the aminopenicillins are metabolized to some extent by hydrolysis of the β-lactam ring. The metabolites are microbiologically inactive. Penicillins and their metabolites are excreted in the urine by tubular secretion. Most of the drug is excreted in the urine within 1 hour of IM injection of sodium or potassium penicillin in aqueous solution. Probenecid competitively inhibits renal tubular secretion of penicillins and can prolong the half-life of penicillin. However, probenecid is rarely used for this purpose in clinical situations.

Summary of Penicillins and Derivatives

Natural Penicillins

The only natural penicillins still in use are penicillin G and penicillin V. Penicillin V has a phenoxymethyl group that provides more acid stability in the stomach, allowing for oral administration. It has been used orally in people, but it is of limited value in animals. It had low oral absorption and limited spectrum in calves (Soback et al., 1987b). In dogs, oral administration of penicillin V tablets produces maximum plasma concentrations of 3.5–4.8 μg/ml, but the concentrations decline quickly and were above 0.5 μg/ml for only approximately 3 hours (Watson et al., 1987a, 1987b).

Penicillin formulations: There are three injectable formulations. Pharmacokinetics of these formulations are provided in Table 33.3:

1) *Na^+ and K^+ salts of penicillin, also called crystalline penicillin:* These are water soluble solutions and may be injected intravenously (IV), intramuscularly (IM), or subcutaneously (SC). They achieve rapid, but short-lived, plasma concentrations.
2) *Procaine penicillin G (Crysticillin, Pen-Aqueous):* This compound is a poorly soluble salt in an aqueous vehicle suspension that is slowly absorbed following intramuscular (IM) or subcutaneous (SC) injection. *Do not administer IV.*
3) *Benzathine penicillin G (Benza-pen, Durapen, Flo-cillin):* This preparation is the so-called "long-acting penicillin". It is absorbed more slowly than the procaine salt because of its insolubility. It produces persistent, but low, plasma concentrations. Most formulations of long-acting penicillin contain 50% procaine penicillin G, and 50% benzathine penicillin G. *Do not administer IV.*

Additional formulations and route of administration are the formulations of penicillin G administered via intramammary routes to treat bovine mastitis.

The doses of penicillin G vary greatly depending on the formulation, the species of animal, and the disease treated. It is best to consult references related to the specific disease being treated. In general, Na^+ or K^+ penicillin G are administered IM, or IV at doses of 20,000 to 50,000 U/kg every 4 to 6 hours. Procaine penicillin G is administered IM or SC at dosages of 22,000 to 70,000 U/kg, every 12 to 24 hours. Treatment of streptococcal infections may use a lower dose, but for some infections such as those caused by *Arcanobacterium* (formerly called *Actinomyces*) doses as high as 100,000 U/kg have been recommended.

The United States FDA–approved label dose for cattle is 7,500 U/kg, but the approved withdrawal time for food animals – 10 days – applies only to an approved food animal dose. Since the doses used in food animals are extralabel, extended withdrawal times must be applied. Food animal residues and withdrawal times are discussed further in Chapter 61 of this book.

Aminopenicillins

Ampicillin and amoxicillin have been used in the treatment of a variety of diseases in domestic animals. The half-life of all aminopenicillins is short (Table 33.2), requiring frequent administration for some infections, particularly gram-negative pathogens that may have high MIC values.

Aminopenicillins are popular because they have a broader spectrum of activity compared to penicillin G, can be administered orally, and are relatively inexpensive and safe. The aminopenicillins differ from penicillin by the addition of an amino group, and amoxicillin has a parahydroxy group that ampicillin lacks. Compared to penicillin G, these compounds have two ionization points (pK_a 2.7 and 7.3). Ampicillin is more soluble than amoxicillin, and is also more lipophilic (Log P ampicillin 1.35; Log P amoxicillin 0.87), but amoxicillin is better absorbed by a factor of approximately two in most animals.

Compared to penicillin G, the aminopenicillins can penetrate the outer layer of gram-negative bacteria better than penicillin G; therefore, they have a spectrum of activity that includes those listed for penicillin but is extended to include some of the gram-negative bacteria (e.g., susceptible Enterobacteriaceae). However, acquired resistance can be common and this group is still quite susceptible to β-lactamase. To overcome resistance the β-lactamase inhibitors clavulanic acid and sulbactam have been added to amoxicillin (Clavamox) and ampicillin (Unasyn), respectively, to increase the spectrum. These are discussed in section β-Lactamase Inhibitors.

Aminopenicillin formulations: The formulations of aminopenicillins used in veterinary medicine (examples of brand names in parentheses) that are available include:

1) *Sodium ampicillin:* This formulation is used for injection IM, IV, SC (Omnipen®).
2) *Ampicillin trihydrate (Polyflex®):* This is a poorly soluble, slow-release aqueous suspension. Absorption of this formulation is erratic, and it produces prolonged, but low, blood concentrations.
3) *Amoxicillin trihydrate:* (Amoxi-inject®).
4) *Ampicillin:* These are oral products such as tablets, capsules, and liquid suspensions (for example, Omnipen®).
5) *Amoxicillin:* These are oral products, such as tablets and liquids (Amoxi-Tabs®, Amoxi-Drops®, and others).
6) *Intramammary preparations:* These include Amoxi-Mast, which is used for treating mastitis.

The elimination of the aminopenicillins is slightly longer than that reported for sodium salts of penicillin, but this difference does not appear to relate to a difference in clinical efficacy. Common doses are listed in Table 33.4, and listed for breakpoints in Table 33.1. A common formulation used in animals is ampicillin trihydrate (Polyflex), a poorly soluble preparation. After IM injection, the half-life is 6.7 hours in cattle, which is adequate for once-daily administration (Gehring et al., 2005). The IM injection route in horses also prolongs the plasma concentration (Table 33.2).

Table 33.4 Common dosages for ampicillin and amoxicillin

Drug	Dose and species
Amoxicillin oral	Dogs, cats 6.6–20 mg/kg q 8–12 h
Ampicillin trihydrate injection	Cattle 6.6–22 mg/kg q 8, 12, or 24 h (24 h most common); the approved label dose in cattle is 4.4–11 mg/kg IM, q 24 h Dogs, cats: 10–20 mg/kg, q 12–24 h, IM, SC
Ampicillin sodium	Horses IV 10–20 mg/kg q 6–8 h
Ampicillin sodium	Horses IM 10–22 mg/kg q 12 h
Ampicillin sodium	Dogs, cats: 10–20 mg/kg, q 8 h, IV, IM, or SC

An important difference between the aminopenicillins and penicillin G is that the aminopenicillins are not inactivated by gastric acid and may be administered orally. There appears to be a saturable transport process for absorption of aminopenicillin in the intestine. Oral absorption of the penicillins in various species and effect of feeding and age is discussed in the Section Absorption.

In addition to the formulations listed above, there are ester derivatives of ampicillin, but these are not currently available. The advantage of these esters is that they are stable in the gastrointestinal tract and are absorbed intact, but esterases release the active drug after absorption across the intestinal mucosa. They are absorbed much better than the parent drugs. Examples of these drugs are *pivampicillin* and *bacampicillin* (Sarasola and McKellar, 1994; Ensink et al., 1996).

Antistaphylococcal Penicillins

Also called the β-lactamase stable penicillins, this group of antibiotics includes the isoxazolylpenicillins (e.g., oxacillin, cloxacillin, and dicloxacillin) and synthetic derivatives of penicillin (e.g., methicillin and nafcillin). These drugs are rarely used clinically in veterinary medicine (except for intramammary forms). These drugs have the disadvantage of poor or inconsistent oral absorption in animals and short half-life. More details on these drugs are provided in earlier editions of this book. They are resistant to the β-lactamase of *Staphylococcus* spp. They have minimal activity against gram-negative bacteria because they do not exhibit good penetration of the outer layer of these bacteria.

One of the drugs in this group is methicillin. If *Staphylococcus* shows phenotypic resistance to methicillin, it is a marker for resistance mediated by the *mecA*-gene, which codes for a resistant PBP protein. When it occurs in *S. aureus*, this type of resistance is called *methicillin-resistant Staphylococcus aureus*–MRSA. If the organism is *Staphylococcus pseudintermedius*, it is referred to as MRSP. Today, oxacillin is used to test for this resistance, even though the resistant strains are still referred to as "methicillin-resistant" (CLSI, 2013).

Preparations available: The only drug from this group that is used clinically is cloxacillin, which is used to treat staphylococcal and streptococcal mastitis. It is also available as an intramammary infusion for dry cows (cloxacillin benzathine).

Extended-Spectrum Penicillins

The extended-spectrum penicillins have also been called the *antipseudomonas penicillins* because they are among the few drugs active against *Pseudomonas*. This group includes the carboxypenicillins – carbenicillin and ticarcillin – because of a substitution of a carboxy group for the amino group on ampicillin, and the ureidopenicillins, which include piperacillin and azlocillin. The carboxy group decreased activity against *Streptococcus*, but improved penetration through the outer cell membrane of gram-negative bacteria. Substitution of a ureido for the carboxy group produced ureido penicillins, which maintained ampicillin's activity against *Streptococcus* and also produced good penetration through the outer membrane of gram-negative bacteria.

The value of this group of penicillins, particularly the ureidopenicillins, is their broad-spectrum activity and that they are able to penetrate the outer wall of *Pseudomonas* and some other gram-negative bacteria (e.g., *Proteus*, *Providencia*, and *Enterobacter*) better than other penicillins. Like the other penicillins, they are susceptible to β-lactamase inactivation, but the addition of tazobactam to piperacillin reduces this inactivation (discussed further in Section β-lactamase Inhibitors). This group has good synergistic activity when administered with the aminoglycosides (e.g., gentamicin, amikacin). The ureido penicillins also have good activity against anaerobic bacteria, and piperacillin may have some activity against enterococci.

Pharmacokinetic features: Despite advantages listed above, the disadvantage of this group of penicillins is the short half-life, which necessitates frequent administration. The half-life of these drugs is approximately 1 hour or less in most animals (VanCamp et al., 2000; Garg et al., 1987; Tilmant et al., 1985). The apparent volume of distribution is between 0.2 and 0.4 l/kg for most drugs in this class, much like other penicillins.

Clinical use: Ticarcillin and ticarcillin–clavulanate (Timentin®) was once a popular injectable antibiotic used in veterinary medicine and there was a formulation used in horses as an intrauterine flush diluted in saline (VanCamp et al., 2000). However, ticarcillin and the combination with clavulanate are no longer commercially available. Likewise, there are no longer formulations of carbenicillin available. More details about both of these drugs can be found in earlier editions of this book.

One drug from this group that still remains commercially available is piperacillin–tazobactam. (Piperacillin as a single agent is not available.) Piperacillin–tazobactam is discussed in more detail with the β-lactamase inhibitors.

Adverse Effects

Penicillins are very safe drugs, with relatively few adverse effects reported. Like most β-lactams, adverse effects are rare. The most common, and often most serious, adverse effects are attributed to allergy – immune-mediated reactions or allergic reactions. These are common in people from penicillins (approximately 15% of the human population) and are seen in veterinary species. Treatment may require administration of medications to attenuate the allergic response and avoidance of future use. Coombs-positive hemolytic anemia has been reported in horses following penicillin administration (Blue et al., 1987; Step et al., 1991).

After oral administration, there can be disruption of intestinal bacteria. *Clostridium* bacterial intestinal overgrowth from oral administration is a risk in guinea pigs, hamsters, gerbils, and rabbits.

Central nervous system reaction can be produced by penicillins (and other β-lactam antibiotics). At high concentrations these drugs can inhibit GABA (an inhibitory neurotransmitter) and cause excitement and seizures. Procaine, which is in some preparations, also causes excitement in some animals (horses) (Neilsen et al., 1988). There may be free procaine in some formulations of aqueous procaine penicillin G. When injected IM (or inadvertently IV) it can elicit an excitatory response. Because procaine can mask pain, and produce excitation in horses, its use is regulated in racing horses (see Chapter 57 on control of drugs in racing animals for additional information). Injections of procaine-penicillin in a horse may cause a positive procaine test reaction at the race track for as long as 2 weeks. Oral administration of ampicillin, amoxicillin, and similar formulations may cause vomiting at high doses.

Special Species Considerations

The penicillins are excreted similarly in all mammalian animals. In general, doses and intervals are similar among mammals, except that in larger species, dose intervals may be longer because of slower renal clearance. Renal clearance can be scaled allometrically to show that the larger body weight is associated with slower renal clearance.

In reptile species, clearance of all penicillin drugs is slow. The half-lives are much longer in reptiles, which allows for infrequent dosing intervals of once every 3–5 days. In birds, renal clearance and metabolic rate is high. This difference results in high doses and frequent intervals. Because of the need for frequent dosing, and because injectable drugs of this group may cause intramuscular pain, these drugs may be impractical in most clinical situations involving birds.

β-Lactamase Inhibitors

The β-lactamase inhibitors are a specific class of drugs with little antibacterial effects of their own, but they act

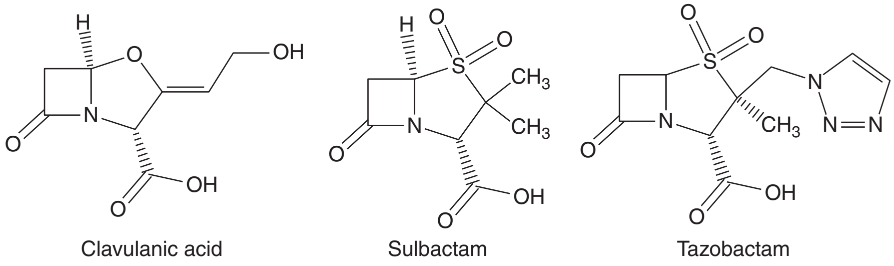

Figure 33.3 β-lactamase inhibitors: Structure of clavulanic acid (left), sulbactam (center), and tazobactam (right).

to inhibit the β-lactamase enzyme. They have structures that resemble the β-lactam antibiotics with an intact β-lactam ring structure (Figure 33.3). They are always combined with another active drug of the β-lactam class. These combinations, particularly amoxicillin–clavulanate, have been popular in veterinary medicine (Mealey, 2001). The primary drugs of this group are clavulanic acid (also called *potassium clavulanate*), combined with amoxicillin, sulbactam (combined with ampicillin), and tazobactam (combined with piperacillin).

Mechanism of Action

The β-lactamase inhibitors produce antibacterial effects only at high concentrations (Díez-Aguilar et al., 2015). They bind to the β-lactamase enzyme that is produced by gram-negative or gram-positive bacteria. This usually is an irreversible, noncompetitive binding. Clavulanate is considered an irreversible, suicide inhibitor. A wide range of β-lactamases are inhibited by clavulanate, including class B β-lactamases, TEM and SHV enzymes found in Enterobacteriaceae, many of the extended-spectrum β-lactamases, and various chromosomally mediated enzymes (Finlay et al., 2003). AMP-C β-lactamases are usually not inactivated by these agents.

When an inactive enzyme complex is formed from these inhibitors, the coadministered antibiotic (e.g., amoxicillin or ampicillin) can exert its antibacterial effect. All β-lactamase inhibitors are not equal with respect to potency and ability to bind β-lactamase enzymes. For example, compared to clavulanate, sulbactam is less active against β-lactamase of *Staphylococcus*, *Bacteroides,* and some *E. coli*. However, whether these differences are important clinically is not known.

Pharmacokinetics

The pharmacokinetics of clavulanate in animals has been studied more than the other inhibitors (Bywater et al., 1985; Vree et al., 2003). Clavulanate is notoriously unstable and should be protected from moisture. Tablets are packaged in foil-protective packaging. Although clavulanate is absorbed orally (the only one of the β-lactamases absorbed orally), the absorption is variable and can vary highly among animals administered similar doses (Vree et al., 2003). There is evidence that high doses of amoxicillin inhibit the absorption of clavulanate in dogs and people (Vree et al., 2003). Clavulanate is susceptible to enzymatic degradation and is excreted by glomerular filtration, whereas amoxicillin is eliminated by renal tubular excretion.

Examples and Clinical Use

Amoxicillin–clavulanic acid (Clavamox®, Synulox): This is one of the most popular oral antibiotics used in small animals. Amoxicillin–clavulanate, sometimes called potentiated amoxicillin and co-amoxiclav, extends the spectrum of amoxicillin to include many of the β-lactamase–producing bacteria. There is an equivalent drug used in people (Augmentin), which is one of the most popular drugs in human medicine. The human drug is not entirely equivalent because the proportion of amoxicillin: clavulanate may be different. Clavamox has a 4 : 1 ratio, whereas augmentin is either in a 4 : 1 ratio or a 7 : 1 ratio, depending on the tablet size. The dose administered to animals is a fixed ratio of 4 : 1 (amoxicillin : clavulanate), but because of lower absorption of clavulanate and more rapid excretion, the ratio in the body can be highly variable and as low as 20 : 1. For susceptibility testing, CLSI (CLSI, 2015) uses a fixed ratio of 2 : 1 (amoxicillin : clavulanate) (Table 33.1).

In small animals Clavamox® has been used to treat infections in almost all tissues (except CNS). It has been successful for skin infections and urinary tract infections (Bywater et al., 1985; Senior et al., 1985; Weese et al., 2011; Hillier et al., 2014). It is particularly useful to treat infections caused by β-lactamase–producing staphylococci. Amoxicillin–clavulanate also is useful for treating anaerobic infections in dogs and cats, such as those of the oral cavity (Indiveri and Hirsh, 1985).

In large animals, amoxicillin–clavulanate has not been an important drug. As reported previously, the oral absorption of amoxicillin in horses is small and unlikely to reach therapeutic concentrations. The oral administration has also been examined in calves (Soback et al., 1987a). There is sufficient absorption of amoxicillin–clavulanate in preruminant calves, but three-times-daily administration was recommended (Soback et al., 1987a), which is impractical. Oral absorption was not high enough in ruminant calves – because of degradation in the rumen – to produce therapeutic concentrations.

Sulbactam–ampicillin (Unasyn®): This is a human drug, but veterinary preparations exist in other countries. This drug has been administered IM, IV, and SC to dogs, cats, horses, and cattle. (Sulbactam is not absorbed orally.) In some countries (e.g., Canada) this combination is used as an intramuscular drug (ampicillin trihydrate + sulbactam, Synergistin) in cattle for the treatment of diseases such as pneumonic pasteurellosis (Risk and Bentley, 1987; Risk and Cummins, 1987; Girard et al., 1987).

Ticarcillin–clavulanic acid (Timentin®): This product is no longer available. Information about this preparation may be found in earlier editions of this book.

Piperacillin–tazobactam (Zosyn®, also referred to as "Pip-Taz"): This is one of the most popular intravenous (IV) antibiotics for in-hospital use in people. Piperacillin is an extended-spectrum penicillin and tazobactam has essentially the same activity as clavulanate. The use includes septicemia, urinary tract infections, skin, soft-tissue, respiratory infections, intraabdominal infections, and gynecological infections. Targeted organisms include bacteria of the Enterobacteriaceae (*Escherichia coli, Klebsiella pneumonia*), including some ESBL-producing strains, and *Pseudomonas aeruginosa*. It also has activity against *Streptococcus* spp. and *Staphylococcus* spp. Formulations are typically in 8 : 1 ratio of piperacillin : tazobactam available in injectable vials reconstituted with sterile water, 0.9% saline solution, or 5% dextrose in water, and further diluted to desired volume for intravenous fluid administration.

The pharmacokinetics have been studied in dogs. The combined analysis of seven different studies showed that it has a half-life of only 0.55 (± 0.11) hours and a volume of distribution of 0.276 (± 0.05) l/kg. The short half-life requires frequent administration (every 6 hours), or as a constant rate infusion (CRI). In immunocompetent animals it may be administered at a dose of 50 mg/kg every 6 hours IV (higher doses are needed in immunosuppressed animals), or as a CRI of 3.2 mg/kg/hour after a loading dose of 2.25 mg/kg. The breakpoint is listed in Table 33.1.

New β-lactamase inhibitor combinations: There are new β-lactamase inhibitor combinations, but there is no record of their use in veterinary medicine. Ceftazidime–avibactam (Avycaz) and ceftolozane–tazobactam (Zerbaxa) are approved for IV treatment of infections in people. They are third-generation cephalosporin/ β-lactamase inhibitor combinations (discussed in Section Cephalosporins) with a spectrum that includes many ESBL-producing bacteria and *Pseudomonas aeruginosa*. Ceftazidime–avibactam also has activity against some carbapenemase-producing *Klebsiella pneumoniae*. These drugs are used in people primarily for complicated urinary tract and intraabdomnial infections caused by bacteria resistant to other drugs.

Cephalosporins

Cephalosporins that are veterinary labeled, as well as drugs approved for humans (e.g., ceftazidime, cefotaxime, cefazolin), have commonly been used in veterinary medicine for many infections, including pyoderma, urinary tract infection, pneumonia, soft-tissue infection, osteomyelitis, and pre- and postsurgical use. They are often considered first-line treatments, often employed empirically for routine outpatient and in-hospital use. One of the advantages of the use of the generic formulations intended for humans is their low cost. For more resistant infections caused by *Pseudomonas aeruginosa* or the Enterobacteriaceae such as *E. coli*, extended-spectrum drugs of the third and fourth generation have been used.

General Pharmacology

The spectrum includes most bacteria susceptible to amoxicillin and ampicillin, but also includes some β-lactamase-producing bacteria, depending on the specific generation of cephalosporin. Many in this class have greater activity against gram-negative bacteria than amoxicillin or ampicillin. In general (exceptions are noted in Section Classification), the cephalosporins owe their usefulness to activity against *Staphylococcus* (β-lactamase–positive, but not methicillin-resistant strains), streptococci (but not enterococci), and gram-negative bacteria, except *Pseudomonas* (exceptions noted in Section Classification). Although cephalosporin antibiotics show activity against some anaerobic bacteria, they are ordinarily *not* considered a drug of choice for the gram-negative anaerobes. The cephamycins (a subclass of cephalosporins), however, have good anaerobic activity.

Cephalosporins contain a 7-aminocephalosporanic acid nucleus, which is composed of a β-lactam ring fused with a six-membered dihydrothiazine ring (see Figure 33.1). Additions of various groups (shown by the asterisk in Figure 33.1) form derivatives with differences in antimicrobial activity, stability against β-lactamases, protein binding, intestinal absorption, metabolism, and toxicity.

The cephalosporin antibiotics are extremely important in veterinary medicine. They were first produced by a fungus isolated from raw sewage from the sea in Sardinia. Although the antibiotic was first isolated from *Cephalosporium acremonium* in 1948, it was not available commercially until 1962. There are now over 30 cephalosporin antibiotics on the market (most on the human pharmaceutical market), but newer ones have been introduced to veterinary medicine. Although the cephalosporins are widely used in many animal species, the extralabel dosages of these drugs in food-producing animals is not allowed in the United States. Regulatory control of antibiotics in the USA is discussed in Chapter 52 and 55 of this book.

Classification

The cephalosporins are broadly classified into first-, second-, third-, and fourth-generation cephalosporins. There is also a new group active against methicillin-resistant staphylococci that has been called a *fifth* generation (e.g., ceftobiprole and ceftaroline) but there is no record of their use in veterinary medicine. This classification system is somewhat arbitrary, depending on when they were synthesized. This classification is largely based on activity against gram-negative bacteria and susceptibility to β-lactamase. Various other classifications of cephalosporins have been proposed (Williams et al., 2001). For this chapter, we retain the classification categories listed by the CLSI (CLSI, 2015) provided in Table 33.5.

First Generation

The first-generation drugs are effective against almost all gram-positive bacteria, except *Enterococcus*, and their activity includes β-lactamase-positive staphylococcus. They also have greater activity against members of the Enterobacteriaceae than penicillin G. Compared to others in this group, cefazolin has the greatest gram-negative activity (Petersen and Rosin, 1995), and it has been grouped with the second-generation drugs in some references based on this activity (Williams et al., 2001). Gram-negative bacteria may develop resistance by inhibiting penetration and by producing β-lactamase enzymes.

Second Generation

In general, these drugs have greater activity against many gram-negative bacteria that are resistant to the first-generation drugs (e.g., resistant *E. coli, Klebsiella, Proteus, Enterobacter*), but are no more active against the gram-positive bacteria. Improved activity against gram-negative bacteria compared to first-generation drugs is attributed to an increased resistance to β-lactamases. Cefoxitin and cefotetan belong to the cephamycin group and have been used clinically because of good activity against anaerobic bacteria (e.g., *Bacteroides fragilis,* and the *Bacteroides fragilis* group). Cefotetan is no longer available in the USA, but cefoxitin is still used in veterinary patients. Cefaclor (Ceclor®), cefprozil (Cefzil®), and cefuroxime axetil (Cefetin®) can be administered orally, but their use has not been reported for small animals.

Third Generation

This group of antibiotics has more activity against gram-negative bacteria than the earlier generations of cephalosporins. Only ceftazidime and cefoperazone have good activity against *Pseudomonas aeruginosa*, with ceftazidime having the greatest activity. For this reason, ceftazidime has been an important drug for some infections in small animals.

The third-generation drugs, in general, are less active against gram-positive cocci, but there is considerable variability in the activity against staphylococci and

Table 33.5 Classification of cephalosporins

First generation		Second Generation		Third generation	
Drug name	**Brand name**	**Drug name**	**Brand name**	**Drug name**	**Brand name**
cephalexin	generic, Keflex[a]	cefamandole	Mandol	cefoperazone	Cefobid
cephalothin	Keflin	cefmetazole	Zefazone	cefotaxime	Claforan
cefadroxil	Cefa-Tabs[a]	cefonicid	Monocid	ceftazidime	Fortaz
cephapirin	Cefadyl	cefprozil	Cefzil[a]	ceftizoxime	Cefizox
cefazolin	Kefzol	cefotetan	Cefotan	ceftriaxone	Rochephin
cephradine	Velosef[a]	cefoxitin	Mefoxin	moxalactam	Moxam
cefaparin	Cefa-Lak and Cefa-Dri	cefuroxime	Kefurox	cefixime	Suprax[a]
		cefuroxime axetil	Ceftin[a]	cefdinir	Omnicef[a]
		cefaclor	Ceclor[a]	ceftiofur	Naxcel
				cefpodoxime proxetil	Vantin[a]
					Simplicef[a]
				Cefovecin	Convenia

[a] Oral drugs.

Fourth-generation drugs not listed include cefquinome (Cobactan) (veterinary drug) and cefepime (Maximime).

streptococci among this group. For example, cefotaxime has the highest activity against streptococci, but others have less activity. Some of the third-generation agents are more active against *Staphylococcus* spp. than cephalexin. There are only three that can be administered orally. Of these, two have been used in veterinary medicine, cefixime (Suprax®) (Lavy et al., 1995), and cefpodoxime proxetil (Simplicef veterinary formulation and Vantin human formulation). Cefixime is no longer available in the USA.

The human-labeled injectable third-generation drugs have been used in veterinary medicine when resistance has been shown to other drugs. An exception is ceftiofur (Naxcel®), which has been used extensively in cattle, pigs, and horses, and is also approved for use in dogs. The activity of the major metabolite, desfuroylceftiofur, is similar to cefotaxime, which is considered a typical third-generation cephalosporin. Cefovecin (Convenia), is an injectable formulation that has an extremely long half-life in dogs and cats compared to other cephalosporins. Specific agents are discussed in more detail in Section Clinical Features and Specific Drugs Used in Veterinary Medicine.

Fourth Generation

The fourth generation of cephalosporins include the human drug cefepime (Maxipime®), and the veterinary drug cefquinome (Cobactan). The use of cefepime has not been reported in veterinary medicine except for some experimental studies. Cefquinome is approved in other countries, but not the USA. These drugs are discussed in more detail in Section Clinical Features and Specific Drugs Used in Veterinary Medicine.

Mechanism of Action

Similar to other β-lactam antibiotics, the cephalosporins bind to PBPs and disrupt the cell wall. They are usually bactericidal and most often bind the PBP-2 and PBP-3.

Pharmacokinetics

The pharmacokinetic features of specific drugs are provided in Table 33.6.

Pharmacokinetics–Pharmacodynamics

Pharmacokinetic–pharmacodynamic (PK-PD) relationships for cephalosporins are the same as for other β-lactam antibiotics discussed in this chapter. Like other β-lactam antibiotics, cephalosporins are considered to be bactericidal in action; they kill bacteria if the drug concentrations are maintained above the MIC for a critical period during the dosing interval (Turnidge, 1998). Thus the important parameter is considered time above MIC (T > MIC). It is the duration of exposure, rather than the magnitude of the concentration above the MIC that determines efficacy of cephalosporins. Dosage regimens for the cephalosporins have been formulated to consider these PK-PD relationships (Craig, 1995, 2001; MacGowan, 2001; Turnidge, 1998). Among the β-lactams, penicillins are not as bactericidal as carbapenems, and cephalosporins are not as bactericidal as penicillins. Therefore, among the β-lactams, cephalosporins should be maintained above the MIC longer than others in this class. Although the optimum time above the MIC has not been determined for most cephalosporins used in companion animals, in humans and laboratory animals the optimum time above the MIC is regarded as approximately 50% of the dosing interval. However, for treating gram-negative infections, maximum bactericidal effect occurs at 60–70% of the dosing interval, and as the duration of the T > MIC increases, improved clinical outcomes are possible. In some experimental studies the T > MIC may be less than 50%. For example, when four cephalosporins were examined to determine the T > MIC necessary for optimum dosing, the T > MIC was 30–40% of the dosing interval for Enterobacteriaceae and *Streptococcus*, but less than 30% for *Staphylococcus*.

Because the half-lives of most cephalosporins in mammals are short, many regimens for cephalosporins use require an administration frequency of three to four times per day. Alternatively, some of the third-generation cephalosporins have long half-lives, and less frequent regimens have been used for some of these drugs (for example cefpodoxime, cefovecin, cefotaxime, and ceftiofur). However, the long half-life for ceftriaxone in people does not occur in animals because of differences in drug–protein binding (Popick et al., 1987).

The dosing regimen that produces the greatest T > MIC is the CRI, and superior efficacy has been reported from CRI regimens rather than intermittent dosing (Zeisler et al., 1992). Constant rate intravenous infusions have also been calculated for some third-generation cephalosporins for dogs (Moore et al., 2000).

Gram-positive organisms are more susceptible to the bactericidal effect of cephalosporins than are gram-negative bacteria. Additionally, since the MICs are lower for gram-positive bacteria, and antibacterial effects occur at concentrations below the MIC for *Staphylococcus* (postantibiotic effect, PAE), longer dose intervals may be possible for infections caused by gram-positive as compared to gram-negative bacteria. For example, cephalexin or cefadroxil have been used successfully to treat staphylococcal infections when administered twice daily (discussed further in Section Clinical Features and Specific Drugs Used in Veterinary Medicine). Some studies have reported efficacy for cephalexin treating staphylococcal pyoderma in dogs with administration of only once daily (although twice-daily

Table 33.6 Pharmacokinetic parameters of selected cephalosporins in domestic species

Drug	Species	Vd[a] (l/kg)	Clearance (ml/kg/min)	Hallf-life (hour)	Reference
Cephapirin	Foals[b]	1.06	18.4	0.7	Brown et al., 1987
	Horses	0.17	10		Brown et al., 1986a
	Cows[c]		12.7		Prades et al., 1988
	Dogs	0.32	8.9	0.42	Cabana et al., 1976
Cephalothin	Horses	0.15	13.6	0.25	Ruoff and Sams, 1985
Cefadroxil	Horses	0.46	7.0	0.77	Wilson et al., 1985
Cefazolin	Foals	0.45	0.4	1.37	Duffee et al., 1989
	Horses	0.27 (0.03)	5.07 (1.23)	0.62 (0.07)	Multistudy[f]
	Calves	0.17	5.8	0.62	Soback et al., 1987c
	Dogs	0.27 (0.13)	2.89 (0.92)	1.04 (0.46)	Multistudy[e]
Cephalexin	Calves	0.32	1.9	2	Garg et al., 1992
	Cows	0.39	10.5	0.58	Soback et al., 1988
	Sheep	0.17	5.0	1.2	Villa et al., 1991
	Dogs	0.92 (Vd/F) (0.48)	3.14 (CL/F) (0.87)	2.74 (1.6)	Multistudy[d]
	Horse[g]	9.92 (Vd/F)	86.4 (CL/F)	1.64	Davis et al., 2005
Cefoxitin	Calves			1.12	Soback, 1988
	Horses	0.12	4.3	0.82	Brown et al., 1986b
Ceftriaxone	Dogs			0.85	Matsui et al., 1984
	Sheep	0.3	3.7		Guerrini et al., 1985
	Calves			1.4	Soback and Ziv, 1988
Ceftazidime	Dogs			0.82	Matsui et al., 1984
	Sheep	0.36		1.6	Rule et al., 1991
Cefoperazone	Calves			0.89	Carli et al., 1986
	Sheep	0.16	2.7		Guerrini et al., 1985
Moxalactam	Calves			2.4	Soback, 1989

[a] Vd, volume of distribution; [b] neonatal; [c] lactating; [d] analysis of 8 studies with oral dosing and 52 observations. Mean (standard deviation) shown; [e] analysis of 4 studies and 35 observations. Mean (standard deviation) shown; [f] analysis of 3 studies and 17 observations. Mean (standard deviation) shown. [g] oral absorption was only 5% in the cephalexin equine study.

administration is recommended to obtain maximum response).

Susceptibility Testing

Breakpoints for susceptibility testing have been approved by CLSI for testing many of the cephalosporins used in veterinary medicine. These breakpoints are based on PK-PD analysis, MIC distributions, and clinical efficacy (Table 33.7).

Tissue Concentrations and Protein Binding

Cephalosporins are relatively polar antibiotics. They are minimally lipid soluble and have poor intracellular penetration. The volume of distribution is generally in the range of 0.2 to 0.3 l/kg and rarely exceeds 0.5 l/kg. However, they have good distribution into the extracellular fluid of most tissues, except prostate and the CNS. They do not reach effective intracellular concentrations. Their ability to penetrate the epithelial lining fluid of the respiratory tract varies among drugs and across species. Specific features of each drug will be discussed in more detail later in this chapter.

Pharmacokinetic–pharmacodynamic-based dosing regimens use plasma concentrations of the unbound drug as the surrogate marker for determining the optimum dose and interval. Only protein-unbound drug is microbiologically active. Protein binding varies across species and among the drugs. Some cephalosporins are highly protein bound, but for others it is low. There are differences between animals and people that affect their use. For example, ceftriaxone has high protein binding of 90–95% in people, which restricts clearance and causes a long half-life (Popick et al., 1987). But the same drug in dogs has protein binding of only 25% at low concentrations to 2% at high concentrations. Cefazolin has high protein binding in people (85%), but low protein binding in dogs (19%), which favors rapid distribution from plasma to interstitial fluid. The most highly protein-bound cephalosporin in animals is cefovecin, which is greater than 99% bound in dogs and cats, but much lower in some other animal species. This property, in addition to other factors, prolongs the plasma concentrations in dogs and cats.

The effect of protein binding on drug distribution was demonstrated for cephalexin and cefpodoxime, two orally administered cephalosporins used in dogs (Papich et al., 2007). Protein binding is higher for cefpodoxime in dogs (>80%), which prolongs the half-life compared to

Table 33.7 Susceptibility breakpoints for cephalosporins (as approved by CLSI 2013, 2015)

Test/report group	Body site	Antimicrobial agent	MIC interpretive criteria (μg/ml) S	I	R	Comments
Dogs	Skin, soft tissue	Cephalexin	≤2	4	≥8	Cephalexin breakpoints were determined from an examination of MIC distribution of isolates, efficacy data, and PK-PD analysis of cephalexin. The dosage regimen used for PK-PD analysis of cephalexin was 25 mg/kg administered every 12 hours orally.
Dogs	Skin, soft tissue, UTI, respiratory	Cefazolin	≤2	4	≥8	Cefazolin breakpoints were determined from an examination of MIC distribution of isolates and PK-PD analysis of cefazolin. The dosage regimen used for PK-PD analysis of cefazolin was 25 mg/kg administered every 6 hours IV in horses and dogs.
Dogs	Wounds, abscesses	Cefpodoxime	≤2	4	≥8	Approved for dogs at a dose of 5–10 mg/kg once daily orally.
Horses	Respiratory, genital tract	Cefazolin	≤2	4	≥8	Cefazolin breakpoints were determined from an examination of MIC distribution of isolates and PK-PD analysis of cefazolin. The dosage regimen used for PK/PD analysis of cefazolin was 25 mg/kg administered every 6 hours IV in horses.
Horses	Respiratory disease	Ceftiofur	≤0.25	–	–	The susceptible only category is used for populations of organisms (usually one species) for which regression analysis (disk vs. MIC) cannot be performed. This breakpoint will permit detection of strains with decreased susceptibility as compared to the original population.
Swine	Lung (SRD)	Ceftiofur	≤ 2	4	≥ 8	Approved for treating swine respiratory disease.
Cattle	Lung (BRD)	Ceftiofur	≤2	4	≥8	Approved for treating bovine respiratory disease.
Cattle	Bovine mastitis	Ceftiofur	≤ 2	4	≥ 8	Mastitis treatment only.

BRD, bovine respiratory disease; SRD, swine respiratory disease; PK, pharmacokinetic; PD, pharmacodynamic; R, resistant; I, intermediate; S, susceptible; UTI, urinary tract infection. Note that some laboratories have used cephalothin as a test for the first-generation cephalosporins such as cephalexin.

cephalexin. The free drug concentration for cefpodoxime in tissue fluid represented the unbound drug fraction in plasma, reflecting the effect of protein binding to restrict drug diffusion from capillaries into tissues. This phenomenon has also been observed in humans (Liu et al., 2002).

Oral Absorption

Many of the cephalosporins are absorbed orally. Cefadroxil and cephalexin of the first-generation group are well absorbed in small animals, but not in large animals. Oral absorption of the ester formulations (cefpodoxime proxetil) is enhanced. This feature will be discussed in more detail for individual drugs in Section Clinical Features and Specific Drugs Used in Veterinary Medicine. For cefadroxil, but not cephalexin, absorption was enhanced somewhat with food (Campbell and Rosin, 1998).

Oral absorption of cephalosporins is generally too low to be effective in horses and ruminants. However, cefadroxil is absorbed better in the foal than in adult horses (Wilson et al., 1985; Duffee et al., 1989). Oral absorption of cephalexin is low in horses (5%) (Davis et al., 2005) but at 30 mg/kg orally q 8 h, concentrations can be maintained above the MIC of highly susceptible bacteria.

Metabolism

Cephalosporins are minimally metabolized by the liver, but degree of metabolism can vary widely among the various drugs. Ceftiofur is transformed almost completely to the metabolite desfuroylceftiofur, which is responsible for its antibacterial efficacy. Most cephalosporins rely on renal elimination.

Elimination

The cephalosporins are eliminated rapidly after systemic administration. The route of elimination is primarily renal, and concentrations in the urine are usually high. This feature makes cephalosporins good choices for treatment of urinary tract infections.

In general, the cephalosporins have half-lives of 1 to 2 hours, but some (particularly the third-generation cephalosporins) may have longer half-lives, which may allow for infrequent dosing. For example, ceftiofur is metabolized to an active metabolite and has a half-life of approximately 3–6 hours in cattle, 4 hours in dogs, and 2.5 hours in horses.

Clinical Features and Specific Drugs Used in Veterinary Medicine

The drugs in the first-generation group have a spectrum of activity that includes staphylococci and streptococci. Resistance among gram-negative bacteria develops easily, primarily from synthesis of β-lactamase enzymes that can hydrolyze these drugs. Resistance has been demonstrated in clinical studies in which samples were collected from dogs and cats (Thungrat et al., 2015; Oluoch et al., 2001; Walker et al., 2000; Cooke et al., 2002). Some older studies may have underestimated resistance because older breakpoints were higher than current values (Table 33.7). Most of the enteric gram-negative isolates are resistant to first-generation cephalosporins because most wild-type bacteria of the Enterobacteriaceae have MIC values above the susceptible breakpoint for cephalexin. The second, third, and fourth generation have higher activity against the gram-negative pathogens. The situations in veterinary medicine in which extended-spectrum cephalosporins are most often used are for treatment of bacterial infections that are resistant to other drugs. The bacteria often identified in these resistance problems have been *Escherichia coli, Klebsiella pneumoniae, Enterobacter* species, *Proteus* species (especially indole positive), and *Pseudomonas aeruginosa*.

First-Generation Cephalosporins

Veterinarians are familiar with the cephalosporins commonly referred to as the *first-generation cephalosporins* represented by the oral drugs cephalexin (Keflex, Rilexine, and generic forms) and cefadroxil (Cefa-Tabs, Cefa-Drops), and the injectable drug cefazolin (generic). Cefadroxil and cephalexin have been the most extensively used of the oral first-generation cephalosporins in dogs. (Older drugs such as cephradine are no longer available.) Cefadroxil is more lipophilic than cephalexin and has the advantage of being better absorbed orally. The differences between cephalexin and cefadroxil were illustrated in the study by Campbell and Rosin (1998) in which they examined the oral absorption of each drug and the influence of food in dogs after 30 mg/kg every 12 hours. Being more lipophilic, cefadroxil was absorbed better when administered with food and attained higher concentrations in plasma. Cephalexin was less influenced by the presence of food. Every 12-hour dosing is appropriate to maintain concentrations above the MIC (Campbell and Rosin, 1998; Papich et al., 2007).

Cefadroxil: Cefadroxil is available as oral suspension (50 mg/ml) and oral tablets (although the availability of some formulations has diminished in recent years). In cats, cefadroxil pharmacokinetics are similar to that in dogs (Chatfield et al., 1984) and it has a half-life of 2.5 to 2.7 hours. Clinical trials in cats showed that cefadroxil was effective for dermal infections with cure rates of 88% at 10–20 mg/kg and 100% cure rate at 20 mg/kg twice daily. Cefadroxil also is used for urinary tract infections in cats at a dose of 20 mg/kg once daily.

In dogs, cefadroxil has been effective for treatment of urinary tract infections, skin infections, and respiratory infections (Chatfield et al., 1984; Angarano and MacDonald, 1989; Barsanti et al., 1985). The dose for which efficacy has been demonstrated for pyoderma has been 22 mg/kg every 12 hours orally for 21 to 30 days, but efficacy at a once-daily dose of 20 mg/kg once daily also has been reported (Scarampella et al., 2000). Cefadroxil at a dose of 22 mg/kg every 12 hours for 21 days was effective in an experimental model of canine cystitis (Barsanti et al., 1985) and has been approved for dogs for the treatment of urinary tract infections.

Cephalexin: Cephalexin is perhaps the most common oral cephalosporin administered to dogs. There is a chewable canine formulation (Rilexine) and approved formulations available in other countries. Human generic formulations also are administered to animals. Pharmacokinetics are listed in Table 33.6, which summarizes the results from several studies. Its oral absorption ranges from 57% to 73–79% (Lavy et al., 1997; Carli et al., 1999). Another study (Wackowiez et al., 1997), reported oral absorption of 91% in dogs. Most methicillin-susceptible *Staphylococcus* spp. and *Streptococcus* spp. are susceptible and cephalexin is appropriate for these infections in dogs and cats. One of the most common uses is staphylococcal pyoderma in dogs, for which there is established efficacy at a dose of 22–25 mg/kg q 12 h, oral. However, as noted above, most wild-type organisms of the Enterobacteriaceae (e.g., *E. coli, Klebsiella* spp.) are resistant. All *Pseudomonas aeruginosa* are resistant.

Oral absorption of cephalexin in cats is about 56%, with a half-life of 2.25 hours (Wackowiez et al., 1997). At usual recommended doses, this will maintain concentrations for pathogens in cats that cause dermal or urinary tract infections with 12-hour dosing. Cephalexin oral absorption in horses is only 5% (Davis et al., 2005), but can be administered at 30 mg/kg q 8 h orally to achieve effective plasma concentrations above 0.5 μg/ml.

Cefazolin: Cefazolin is the injectable cephalosporin administered often to companion animals. It is inexpensive, has a broad spectrum of activity, and is stable after reconstitution for 1 week if refrigerated (Bornstein et al., 1974). It has been administered IV, IM, and SC to dogs. There are several papers that have examined its activity and pharmacokinetics in animals (Petersen and Rosin, 1995; Rosin et al., 1989, 1993; Dickson et al., 1987; Marcellin-Little et al., 1996). Pharmacokinetics are shown in Table 33.6. It is more active against *E. coli* than cephalothin or cephalexin, and after standard doses of 20–22 mg/kg IV, concentrations can be maintained during surgical procedures. Cefazolin has low plasma protein binding (19% in dogs, which is much lower than in humans) and diffuses into tissue fluid to reach concentrations that parallel those in plasma (Rosin et al., 1989, 1993). Cefazolin also penetrated normal and osteomyletic bone in concentrations similar to plasma concentrations (Daly et al., 1982). Distribution was not impaired in osteomyletic bone. This advantage of good penetration has allowed it to be used for prevention and treatment of bone infections (Daly et al., 1982) and as a common antibiotic to use prophylactically prior to orthopedic surgery (Rosin et al., 1993). Richardson et al. (1992) showed that at a dose of 22 mg/kg IV every hour, cefazolin concentrations in bone were above the MIC_{90} for pathogens causing common postoperative infections. Concentrations in bone of dogs paralleled the plasma concentrations and the optimum dose for orthopedic surgery was determined by Marcellin-Little et al. (1996). To maintain cefazolin concentrations above 20 μg/ml (10× the MIC_{90} of susceptible organisms a dose of 22 mg/kg administered IV every 2 hours or 8 mg/kg administered IV every hour was determined. During surgery, disease, anesthesia, and blood loss may affect distribution and clearance of some drugs. However, when cefazolin was administered to dogs with hemorrhagic shock, the clearance was slower, but it was offset by an increased volume of distribution (Dickson et al., 1987). Consequently, the plasma concentrations were not different in dogs when compared before and after shock. Some cephalosporins affect blood clotting and platelet function in animals and may be risky to use prior to surgery. However, when cefazolin was compared to cephalothin and cefmetazole in dogs, the investigators showed that cephalothin decreased platelet aggregation, and cefmetazole prolonged bleeding time (Wilkens et al., 1994), but cefazolin caused no adverse effects on platelet aggregation, bleeding time, platelet count, platelet size, or bleeding times.

Cefazolin is used occasionally in horses as an injectable preoperatively or perioperatively. The current doses are derived from pharmacokinetics and susceptibility data (Table 33.6, 33.7). Cefazolin has a slower terminal half-life from IM than from IV administration. The IM injection is thought to have a longer half-life because of slower absorption from muscle (caused by the flip-flop effect). Subsequent doses of 10 to 20 mg/kg can be administered q 8 h IM or q 6 h IV.

Cephapirin: Cephapirin is not used very often for systemic use in animals, but there are dry cow and lactating cow preparations (Cefa-Dri®, Cefa-Lak®, respectively) of cephapirin for intramammary infusion. Cephapirin is used for treatment of mastitis caused by *Streptococcus* or *Staphylococcus*. Cephapirin benzathine is used for dry cow treatment 300 mg/10 ml, administered in each quarter at the time of drying. Cephapirin sodium 200 mg/10 ml is infused 200 mg to each affected quarter every 12 hours.

Second-Generation Cephalosporins

Of the second-generation cephalosporins, the one used most often in veterinary medicine is cefoxitin (Petersen and Rosin, 1993). Cefotetan was once used, but is no longer available commercially. The use has been valuable for treating organisms resistant to the first-generation cephalosporins or in cases in which there are anaerobic bacteria present. Anaerobic bacteria such as those of the *Bacteroides fragilis* group can become resistant by synthesizing a cephalosporinase enzyme, but cefoxitin and cefotetan, which are in the cephamycin group, are resistant to this enzyme. Therefore, this group has been valuable for some cases such as septic peritonitis that may have a mixed population of anaerobic bacteria and gram-negative bacilli.

There are no reports of clinical use of oral second-generation cephalosporins in small animals, but doses have been extrapolated from human studies. Cefaclor was shown to have 75% oral bioavailability in dogs and there are anecdotal accounts of its use (Waterman and Scharfenberger, 1978). Interstitial drug levels were lower than serum, but urine concentrations were high for 4 hours after dosing.

Third-Generation Cephalosporins

The third-generation cephalosporins are the most active of the cephalosporins against gram-negative bacteria,

Table 33.8 Dose, formulations and indications for ceftiofur in animals

Species	Formulation	Dose
Cattle	Ceftiofur crystalline free acid (Excede)	6.6 mg/kg, with a single SQ injection in the middle third of the posterior aspect of the ear.
Horses and Foals	Ceftiofur crystalline free acid (Excede)	6.6 mg/kg IM in neck muscle (15 ml per 1,000 pounds). Administer a second dose in 4 days. Do not administer more than 20 ml in one site.
Pigs	Ceftiofur crystalline free acid (Excede)	5.0 mg/kg IM injection in the postauricular region of the neck.
Cattle	Ceftiofur hydrochloride (Excenel)	1.1–2.2 mg/kg q 24 h for 3 days IM or SQ. Intrauterine (retained fetal membranes): 1 g ceftiofur diluted in 20 ml sterile water infused in uterus once at 14–20 days after calving. Treatment of postpartum metritis: 2.2 mg/kg once daily for 5 days SQ or IM.
Pigs	Ceftiofur hydrochloride (Excenel)	3–5 mg/kg q 24 h for 3 days IM.
Cattle	Ceftiofur sodium (Naxcel)	Bovine respiratory disease (BRD): 1.1–2.2 mg/kg (0.5–1.0 mg/pound) q 24 h for 3 days IM. Additional doses may be given on days 4 and 5 if necessary. In cattle, these doses also may be administered SQ, which is bioequivalent.
Horses	Ceftiofur sodium (Naxcel)	2.2–4.4 mg/kg q 24 h IM or 2.2 mg/kg q 12 h IM for as long as 10 days. Treatment of some gram-negative infections may require doses at the higher range and up to 11 mg/kg/day IM has been given to horses. Foals: 5 mg/kg q 12 h IV, or CRI of 1 mg/kg/hour IV.
Pigs	Ceftiofur sodium (Naxcel)	Respiratory infections: 3–5 mg/kg (1.36–2.27 mg/pound) q 24 h for 3 days IM.
Sheep, goats	Ceftiofur sodium (Naxcel)	1.1–2.2 mg/kg (0.5–1.0 mg/pound) q 24 h for 3 days IM, SQ. Additional doses may be given on days 4 and 5 if necessary.
Dogs	Ceftiofur sodium (Naxcel)	Urinary tract infection: 2.2 to 4.4 mg/kg q 24 h SQ. Dose not established for cats but has been extrapolated from canine dose.

especially enteric organisms that are resistant to other cephalosporins. The injectable drugs are administered IV, SC, or IM. The SC route is often used for convenience (Moore et al., 2000; Guerrini et al., 1986). Veterinarians have observed that the IM or SC administration of some of these drugs can be irritating and painful. Cefotaxime (Claforan, and generic) is one of the typical members of this group to which others are compared. It has activity against most enteric gram-negative bacteria and some streptococci. Except for a few pharmacokinetic studies (Guerrini et al., 1986; McElroy et al., 1986), there are no published studies in which cefotaxime has been evaluated in veterinary patients. However, the pharmacokinetics between dogs and humans are similar enough that doses, as well as clinical uses, have been extrapolated from human medicine. Generally, cefotaxime is administered IV, IM, or SC to dogs and cats at a dose of 30 mg/kg every 8 hours. When administered SC to dogs, and IM to cats, the absorption was high (McElroy et al., 1986; Guerrini et al., 1986).

Ceftiofur: Ceftiofur (Naxcel, Excenel, Excede) has been approved for veterinary use for many years. It is unique because it is not used in human medicine. It is available in three forms: (i) ceftiofur sodium – Naxcel, (ii) ceftiofur hydrochloride suspension (Excenel), and (iii) ceftiofur crystalline free acid (Excede). After injection it is converted to an active metabolite, desfuroylceftiofur. The differences in activity between ceftiofur and its metabolite were reported by Salmon et al. (1996). Ceftofur has greater activity than the desfuroyl metabolite against *Staphylococcus* spp. (four to eight times difference) and slightly greater activity against *Streptococcus* spp. Both the parent drug and metabolite are highly active against gram-negative bacteria that cause bovine and porcine respiratory disease, but less so against gram-negative bacteria of the Enterobacteriaceae (MIC 0.5–1 μg/ml). The CLSI (CLSI, 2015) breakpoint for ceftiofur use in cattle and swine is ≤2.0 μg/ml (Table 33.7), but lower for the use in horses. Doses are listed in Table 33.8.

For dogs ceftiofur sodium is approved only for treating urinary tract infections caused by gram-negative bacilli of the Enterobacteriaceae at a dose of 2.2 mg/kg SC once a day. Bacteria with higher MIC values may require larger doses or more frequent administration (Brown et al., 1995). The use of ceftiofur for treating systemic infections in small animals has not been reported; but, on the basis of the pharmacokinetic profile of plasma concentrations (Brown et al., 1995), it appears that the frequency of administration should be greater than once a day to maintain the drug concentrations above the MIC for a sufficient duration. In dogs, anemia and thrombocytopenia are possible if ceftiofur is administered at doses of three times and five times the registered dose of 2.2 mg/kg.

Ceftiofur is the most frequently used cephalosporin in horses. Ceftiofur was approved for use in horses for treatment of respiratory tract infections caused by *Streptococcus equi* subsp. *zooepidemicus* at a dose of 2.2 to 4.4 mg/kg q 24 h IM. Higher doses or more frequent intervals have been recommended for treating gram-negative organisms (e.g., *Klebsiella, Enterobacter, Salmonella*). Because these organisms are inherently more resistant, higher plasma concentrations are needed for efficacy. Studies in foals indicated that a dose of 2.2 to 6.6 mg/kg could be given to foals IM or IV q 12 h for treatment of neonatal sepsis. Based on pharmacokinetic studies (Jaglan et al., 1994) a dose of 4.4 mg/kg injected q 12 h will produce plasma concentrations above the MIC to meet the criteria for effective therapy. Toxicity studies have shown that horses tolerate ceftiofur doses up to 11 mg/kg/day IM, with pain at the injection site and decreased feed consumption as the most common adverse effects at the highest dose.

An important use of ceftiofur is for respiratory and other infections in cattle and pigs. An advantage of ceftiofur is that concentrations quickly fall below the allowed tolerance and withdrawal times for slaughter and milk are short compared to other antibiotics. Use of this drug in food animals is discussed further in Chapter 52 of this book. It has been used for treating bovine respiratory disease (BRD) in cattle and swine respiratory disease (SRD) in pigs. It has activity against bovine and swine respiratory pathogens such as *Actinobacillus pleuropneumoniae, Mannheimia haemolytica, Histophilus somni, Salmonella choleraesuis, Haemophilus*, and *Streptococcus*. This formulation also is approved for treating foot rot in cattle (interdigital necrobacillosis) caused by *Fusobacterium necrophorum, Porphyromonas levii,* and *Bacteriodes melaninogenicus*. It is approved for treatment of acute metritis in dairy cattle via a two-dose regimen. The crystalline-free acid (Excede) is a slow-releasing drug that is injected at the base of the ear in cattle and in the neck of pigs. Ceftiofur hydrochloride and ceftiofur crystalline-free acid have also been administered intramammary to dairy cattle (Spectramast).

Ceftazidime: Compared to other cephalosporins, ceftazidime is the most active against *Pseudomonas aeruginosa*, against which all the other cephalosporins, except cefoperazone, have little or no activity. Ceftazidime has been studied in dogs (Moore et al., 2000; Matsui et al., 1984; Acred, 1983) and it has a short half-life (less than 1 hour) and volume of distribution similar to that in humans. Dosages have ranged from 20–30 mg/kg every 12 hours for Enterobacteriaceae, to 30 mg/kg administered every 4 hours for *Pseudomonas aeruginosa* (Moore et al., 2000).

In vitro activity of ceftazidime is good against most gram-negative bacilli (Martin Barrasa et al., 2000). Isolates of *Pseudomonas aeruginosa* from otitis media showed that 97% were susceptible to ceftazidime (Colombini et al., 2000). In a study that isolated *Pseudomonas aeruginosa* from the skin and ears of dogs, a similar pattern of susceptibility was reported (Petersen et al., 2002). In a study that examined 183 isolates of *Pseudomonas aeruginosa* from various sites in dogs (1993–2000), Seol et al., 2002), 77% were susceptible to ceftazidime.

Because of the good activity against Enterobacteriaceae and *Pseudomonas aeruginosa*, ceftazidime has been used in exotic and zoo animals. In a killer whale, the half-life was greater than 6 hours after IM administration and therapeutic concentrations were maintained after doses of 20 mg/kg every 24 hours, IM (unpublished observations by the author, MGP). In reptiles, cephalosporins are excreted slowly. Ceftazidime pharmacokinetics in sea turtles determined that a half-life of 20 hours allowed for dosing of 20 mg/kg as infrequently as every 72 hours (Stamper et al., 1999). In Eastern box turtles the half-life was 42 hours, which allows for a dose of 20 mg/kg IM every 5 days to maintain concentrations above a therapeutic range (author's data; not yet published).

Cefovecin: In December 2006, cefovecin (Convenia) was introduced to small animal medicine in Europe. The same drug and formulation were available in Canada in October 2007 and in the US in 2008. Pharmacokinetic studies have shown the unique differences between cefovecin and other cephalosporins in dogs and cats (Stegemann et al., 2006b, 2006c). Efficacy studies and clinical field trials have shown its efficacy (Stegemann et al., 2007a, 2007b; Passmore et al., 2007). In the clinical studies, cefovecin was compared to another active antimicrobial (cefadroxil, cephalexin, or amoxicillin–clavulanate) and was found noninferior to these other drugs.

In dogs and cats, cefovecin is approved for treatment of skin and soft-tissue infections. In some countries it is also registered for urinary tract infections. The approved label dose in the US allows for a repeat injection at 7 days at a dose of 8 mg/kg SC. However, concentrations are maintained against some bacteria for 14 days, and the approved labeling in Canada and Europe lists a 14-day dose interval. The studies published show efficacy with a 14-day interval for administration. The injection may be repeated if longer than 14 days is needed for a cure (e.g., canine pyoderma).

The long duration of cefovecin is attributed to the long half-life in dogs and cats. Cefovecin is >99% protein bound in cats and >98% in dogs. With such a small fraction unbound (fu) there is little drug available for excretion and some tubular reabsorption may also occur. Subsequently, the terminal half-life is approximately 7 days in cats and 5 days in dogs. Effective concentrations can be maintained in the tissue fluid for a 14-day interval or longer (Stegemann et al., 2006b, 2006c). Cefovecin is classified as a third-generation cephalosporin and has a low MIC values for many bacteria. Against pathogens from Europe and the US (Stegemann et al., 2006a), cefovecin MIC_{90} values were 0.25 μg/ml for *Staphylococcus pseudintermedius* compared to 2 μg/ml for cephalexin and cefadroxil. It has greater activity against gram-negative bacteria than first-generation cephalosporins, as was demonstrated by the MIC_{90} values of 1 μg/ml compared to 16 μg/ml for cephalexin and cefadroxil. Many other MIC comparisons are provided in the tables in the paper by Stegemann et al. (2006a). But compared to other third-generation cephalosporins such as cefotaxime, it is not as active against gram-negative bacteria of the Enterobacteriaceae.

Oral Third-Generation Cephalosporins

Because the drugs mentioned above are all injectable, there has been a need for an oral extended-spectrum cephalosporin. Cefixime (Suprax) was once used in small animals, but is no longer available (Lavy et al., 1995; Bialer et al., 1987).

Cefpodoxime proxetil: Cefpodoxime proxetil is the oral third-generation cephalosporin used most often in veterinary medicine. It is a prodrug ester (Borin, 1991) that is designed to remain stable in the stomach, but the prodrug is converted to the active cefpodoxime by intestinal brush border enzymes. As a lipophilic ester, it is anticipated that oral absorption will be enhanced if the drug is administered with food, which has been confirmed in people, but not specifically reported for dogs. Cefpodoxime has similar gram-negative in vitro activity as cefixime, but greater activity against *Staphylococcus*.

In dogs pharmacokinetics have been studied to show good oral absorption and a long half-life (4.7 and 5.6 hours) compared to other cephalosporins that allow for once-daily administration at 5–10 mg/kg (Brown et al., 2007; Klesel et al., 1992; Papich et al., 2007). Cherni and colleagues (2006) reported that cefpodoxime proxetil administered once a day (5 mg/kg) to dogs with pyoderma was as effective as twice-daily (26 mg/kg) administration of cephalexin (Cherni et al., 2006). In horses, cefpodoxime proxetil oral absorption was good enough that a dose of 10 mg/kg q 6–12 h produced plasma concentrations that would potentially treat infections in horses (Carrillo et al., 2005), but clinical use in horses has not been reported.

Fourth-Generation Cephalosporins

The only fourth-generation cephalosporin available in the US at this time is cefepime (Maxipime), which is approved for people and occasionally used in animals. It is unique because of its broad spectrum of activity that includes gram-positive cocci, enteric gram-negative bacilli, and *Pseudomonas aeruginosa*. It has the advantage of activity against some extended-spectrum β-lactamase (ESBL)-producing strains of *Klebsiella* and *E. coli* that have become resistant to other β-lactam drugs and fluoroquinolones. Except for investigations in dogs, adult horses, and foals, the use of cefepime has been limited in veterinary medicine (Gardner and Papich, 2001). In the study in dogs, there was a short half-life of 1 hour, and to maintain drug concentrations above an MIC value of 8 μg/ml for 67% of the dosing interval, a dose of 40 mg/kg IV every 6 hours would be necessary. However, this dose would maintain the concentration above an MIC of 2 μg/ml for 100% of the dosing interval and bacteria with lower MIC values (MIC ≤2 μg/ml) could be treated with longer dose intervals. In foals and mares this drug possibly could be used for infections resistant to other drugs. A cefepime dose for foals of 11 mg/kg IV q 8 h (Gardner and Papich, 2001) and for adults of 2.2 mg/kg IV q 8 h (Gardner and Papich, 2001; Guglick et al., 1998) is recommended. When cefepime was administered to horses orally, signs of colic were observed (Guglick et al., 1998).

Cefquinome (Cobactan) has been licensed for use in cattle and horses in Europe since 1994. It is not approved for use in the USA. It is approved for treatment of infections in horses and cattle caused by *Streptococcus equi subsp. zooepidemicus*, septicemia caused by *Escherichia coli*, and respiratory diseases caused by *Pasteurella multocida* and *Mannheimia haemolytica*, digital dermatitis, infectious bulbar necrosis and acute interdigital necrobacillosis (foul in the foot). It is also approved as an intramammary product for treatment of mastitis.

Adverse Reactions

Cephalosporins have a high therapeutic index and have been administered to small animals safely. Some of the adverse reactions are listed in the following sections.

Hypersensitivity Reactions

Hypersensitivity allergic reactions (type I, II, or III) have been observed in small animals after administration, but they are infrequently reported. There appears to be some cross-sensitivity with penicillins, but the incidence has not been reported. One should not assume that, if animals are sensitive to penicillin drugs, they will have adverse effects from cephalosporins. Sensitivity to penicillins may increase the risk of sensitivity to cephalosporins by a factor of 4 (Kelkar and Li, 2001), but many patients who are sensitive to penicillins can receive cephalosporins safely. Cephalexin has a side chain identical to that of amoxicillin, so animals with sensitivity to ampicillin should be administered cephalexin cautiously. Likewise, cefadroxil has the same identical side chain as amoxicillin.

Gastrointestinal

Some dogs vomit after receiving oral cephalosporins (e.g., cefadroxil, cephalexin), particularly at high doses. Dogs also may vomit after rapid injections of intravenous cephalosporins (Petersen and Rosin, 1993). In clinical studies with oral cephalosporins, vomiting and diarrhea are the most common adverse reaction (Frank and Kunkle, 1993). Cephalexin and cefadroxil were the third and fifth most common oral drugs to cause adverse events in dogs according to one survey (Kunkle et al., 1995). In this survey, the most common adverse effects associated with oral cephalosporins were gastrointestinal (vomiting, diarrhea, and loss of appetite). It is believed to be caused by irritation to the stomach mucosa, but the exact mechanism has not been investigated.

Blood Disorders

Cephalosporin-induced hemolysis has been reported in people (Ehmann, 1992). Such a disorder has not been reported from use of cephalosporins in small animals. A positive Coombs test reaction can occur with patients receiving cephalosporins, but it is not associated with hemolytic anemia. High doses of ceftiofur in dogs can cause anemia and thrombocytopenia.

Bleeding Disorders

Bleeding disorders have been reported with some cephalosporins in humans because they may produce a prolongation of the prothrombin bleeding time. Even though this effect can be demonstrated in experimental dogs, it has not been reported to be a clinical problem in veterinary medicine, probably because it is associated only with a few of the cephalosporins that are rarely used in animals. Cephalothin was shown to prolong mucosal bleeding times and adenosine diphosphate (ADP)-induced platelet aggregation in dogs, but did not affect platelet numbers or platelet aggregation from collagen (Schermerhorn et al., 1994). This is a moot point for cephalothin because it is no longer used clinically in dogs. Cefazolin is often administered to dogs, cats, and horses. When it was compared to cephalothin and cefmetazole in dogs, the investigators showed that cephalothin decreased platelet aggregation, and cefmetazole prolonged bleeding time (Wilkens et al., 1994). However, cefazolin caused no adverse effects on platelet aggregation, bleeding time, platelet count, platelet size, or bleeding times.

In people, only the cephalosporins with NMTT (*N*-methylthiotetrazole) side chains are prone to producing bleeding problems. *(The NMTT drugs include cefoperazone, cefotetan, and cefamandole.)* Bleeding problems appear to be related to vitamin K antagonism and/or platelet dysfunction.

Glycosuria

Cephalosporins may cause a false-positive glucose test on a urine sample test, but this occurs only if the test employs the copper-reduction test. Others such as the glucose enzymatic tests are not affected. This is of little clinical significance.

Special Species Considerations

In zoo hoofstock, ceftiofur and other cephalosporins are important injectable drugs. Pharmacokinetics are similar as in other large animals, and doses for the cephalosporins are similar among the large zoo species. Drugs such as ceftiofur crystalline free acid are important in these animals because they can be treated without the need for frequent injections.

In reptiles, cephalosporins are excreted slowly. Ceftazidime pharmacokinetics in sea turtles determined that a half-life of 20 hours allowed for dosing of 20 mg/kg as infrequently as every 72 hours (Stamper et al., 1999). Regimens for cephalosporins in other reptiles have been published that also allow for long dosing intervals (Jacobson, 1999).

In birds, rapid elimination and poor oral absorption are a problem. This requires high doses and frequent administration for cephalosporins (Flammer, 1998). Doses for cephalexin and cefotaxime in birds has been listed as high as 100 mg/kg, q 8 h.

Carbapenems (Penems)

Carbapenems (also called *penems*) include imipenem, doripenem, ertapenem, and meropenem. They have the broadest antibacterial action in comparison to other β-lactams, even surpassing third-generation cephalosporins. The carbapenems have become valuable antibiotics because of a broad spectrum that includes many bacteria resistant to other drugs (Edwards and Betts, 2000). Carbapenems are not active against methicillin-resistant staphylococci or resistant strains of *Enterococcus faecium*. The high activity of the carbapenem group of β-lactams is attributed to its stability against most of the β-lactamases (including ESBL) and ability to penetrate porin channels that usually exclude other drugs (Livermore, 2001). Resistance to carbapenems has been extremely rare in veterinary medicine, but as discussed earlier carbapenemase-producing bacteria have been identified in animals (Abraham et al., 2014).

The carbapenems have been used primarily for serious, resistant infections that would otherwise require multiple drugs, including aminoglycosides. They are more bactericidal than other β-lactam antibiotics against gram-negative bacteria because they affect PBP-1 and PBP-2 and produce postantibiotic effects (PAE) that are not seen with other β-lactams. The rapid bactericidal activity is less likely to induce release of endotoxin in patients from gram-negative sepsis during treatment. The bactericidal activity can be maintained with a shorter time above the MIC than other β-lactam antibiotics (Turnidge, 1998). In veterinary medicine, their use has been limited to serious infections caused by bacteria resistant to other antibiotics. Imipenem and meropenem are the most commonly used of this group. The breakpoints for susceptibility testing are not established for animals. For humans the susceptible breakpoint is ≤1 μg/ml for Enterobacteriaceae (≤0.5 μg/ml for ertapenem) and ≤2 μg/ml for *Pseudomonas aeruginosa.*

Imipenem (Primaxin®)

Imipenem has been used occasionally for treating serious infections in veterinary medicine. Imipenem is ordinarily metabolized extensively by the renal tubules (a brush border enzyme) to a potentially toxic compound. The drug cilastatin inhibits the renal enzymes and imipenem is combined with cilastatin in the product Primaxin® to avoid renal toxicity and to achieve high urine concentrations of active drug.

Some disadvantages of imipenem are the inconvenience of administration, short shelf-life after reconstitution, and high cost. It must be diluted in fluids prior to administration. A common dose for small animals is 10 mg/kg q 8 h or 5 mg/kg q 6 h. This dose must be given by constant rate infusion over 30–60 minutes, but it has been administered subcutaneously. One of the adverse effects caused from imipenem therapy is seizures. Another problem is the risk of renal injury, which should be minimized by the addition of cilastatin (Barker et al., 2003).

Meropenem (Merrem®)

Meropenem is a newer-generation carbapenem. It has antibacterial activity approximately equal to, or greater than, imipenem. Its advantage over imipenem is that it is more soluble and can be administered in less fluid volume and more rapidly. For example, small volumes can be administered subcutaneously with almost complete absorption. There also is a lower incidence of adverse effects to the central nervous system, such as seizures (Edwards and Betts, 2000). Based on pharmacokinetic experiments (Bidgood and Papich, 2002), the recommended dose in dogs for Enterobacteriaceae and other susceptible organisms is 8.5 mg/kg SC every 12 hours, or 24 mg/kg IV every 12 hours. For infections caused by *Pseudomonas aeruginosa*, or other similar organisms that may have MIC values as high as 1.0 μg/ml, the dose is 12 mg/kg q 8 h, SC, or 25 mg/kg q 8 h, IV. For susceptible organisms in the urinary tract, 8 mg/kg, SC, every 12 hours can be used. In the experience of the author, these doses have been well-tolerated except for slight hair loss over some of the SC dosing sites. The dose for cats, based on pharmacokinetic studies, is 10 mg/kg twice daily, SC, IM, or IV.

Ertapenem (Invanz)

Ertapenem is one of the newest of the carbapenems. Ertapenem has good activity against most gram-negative organisms, except *Pseudomonas aeruginosa.* It has a longer half-life in people, allowing for once-daily administration. However, in dogs the protein binding was only 46% and the half-life is not prolonged as it is in people. The half-life after a SC injection of 20 mg/kg was 1.3 hours with high systemic clearance. A dose of 30 mg/kg every 12 hours SC in dogs will maintain concentrations in the therapeutic range for dogs. The dose should be increased to every 8 hours in immunocompromised animals.

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34

Tetracycline Antibiotics

Mark G. Papich and Jim E. Riviere

General Pharmacology of Tetracyclines

The tetracycline antibiotics were initially discovered in 1944, with the first one being chlortetracycline. These were isolated from the *Streptomyces* species (*S. rimosus* and *S. aureofaciens*), and later expanded to include the various semisynthetic products that include tetracycline, doxycycline, and minocycline. Oxytetracycline was discovered in 1948, tetracycline in 1953, doxycycline in 1967, and minocycline in 1972. The newest development is the glycylcyclines, which are derivatives of minocycline. Tigecycline is the only available representative of this group, which possesses better antimicrobial activity than older drugs (Agwuh and MacGowan, 2006). Tigecycline use in veterinary medicine has not been reported.

The tetracyclines are a group of four-ringed amphoteric compounds (Figure 34.1) that differ by specific chemical substitutions at different points on the rings. As a group, the tetracyclines are acidic, hygroscopic compounds in aqueous solutions and easily form salts with acids and bases, with which they are commonly formulated. The most common salt form is the hydrochloride formulation, as is the case with oxytetracycline. However, some tetracyclines, especially oxytetracycline, are formulated with vehicles (excipients) to prolong absorption from the injection site. Some of the chemical and physical properties of the tetracyclines used in veterinary medicine today are listed in Table 34.1.

Tetracyclines have activity against both gram-positive and gram-negative bacteria, but resistance occurs frequently. They also have activity against atypical pathogens such as *Mycoplasma*, blood-borne pathogens (hemoplasma), and organisms such as *Rickettsia* transmitted by ticks and other parasites. Clinically accepted indications include abscesses, enteritis, *Leptospirosis*, pneumonia, bovine and swine respiratory disease, pododermatitis, treatment of tick-borne pathogens, skin and soft tissue infections, canine heartworm disease, and uterine infections.

Many formulations have been administered in medicated water and for feed for production purposes (growth promotion). The US Food and Drug Administration (FDA) announced that as of 2017 the production uses of these antibiotics will be voluntarily withdrawn from livestock use. The tetracyclines comprise the largest group of antibiotics affected by this FDA order. The FDA believes that production use indications such as “increased rate of weight gain” or “improved feed efficiency” are no longer appropriate for the approved conditions of use for medically important antimicrobial drugs. These regulatory changes are provided in the Guidance for Industry (GFI) documents #209 and #213, which may be obtained from the FDA. These medications ordinarily added to feed and water of livestock will not be considered in this chapter because of their future status, and the levels administered are considered subtherapeutic. In addition, the pharmacokinetics of these formulations in the target species is incomplete.

Mechanism of Action

Tetracyclines possess antimicrobial activity by binding to the 30S ribosomal subunit of susceptible organisms. After binding to the ribosome, the tetracyclines interfere with the binding of aminoacyl-tRNA to the messenger RNA molecule/ribosome complex, thereby interfering with bacterial protein synthesis in growing or multiplying organisms (Gale and Folkes, 1953; Suzuka et al., 1966). Tetracyclines require an energy-dependent process to enter bacteria. One of the reasons for their selectivity against microorganisms is that mammalian cells lack this transport mechanism. Tetracyclines also have less affinity for mammalian ribosomes than bacterial ribosomes. Because the binding to the ribosome target is a reversible process, the drug concentrations must be maintained throughout the dose interval and these drugs are generally considered bacteriostatic. Details are described in Section Pharmacokinetic–Pharmacodynamic Properties.

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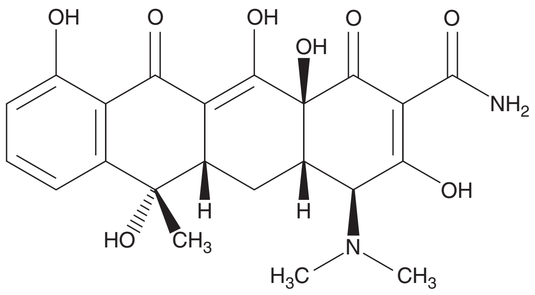
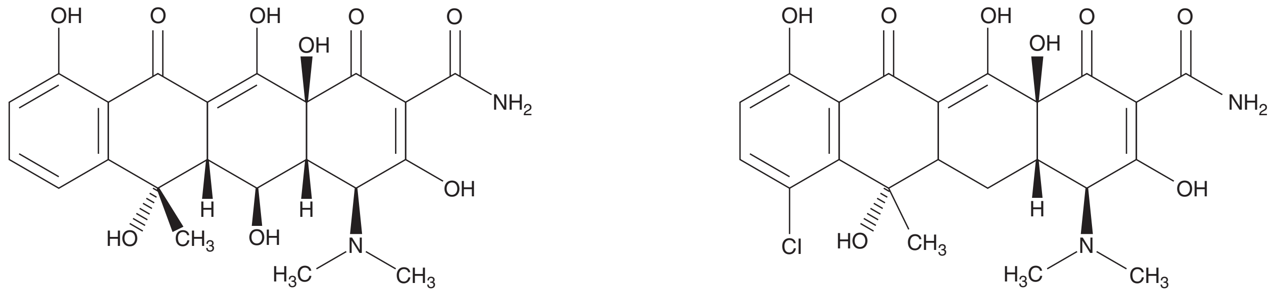
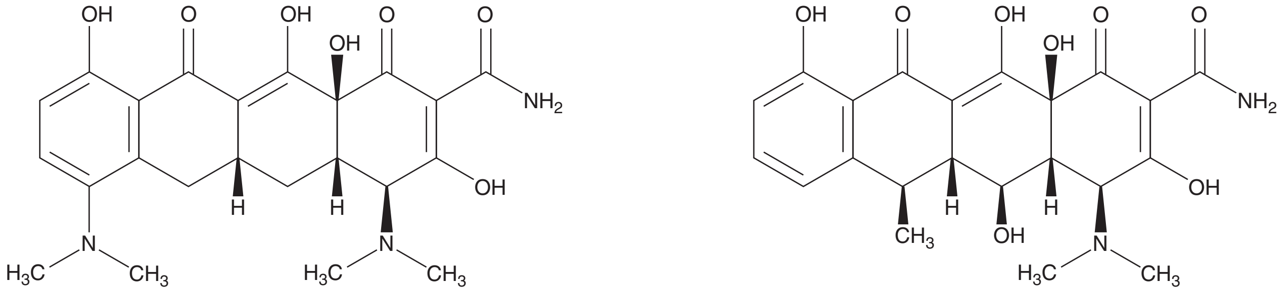


Tetracycline



Oxytetracycline

Chlortetracycline



Minocycline

Doxycycline

Figure 34.1 Tetracyclines and other tetracycline structures.

Resistance: Because the tetracyclines have been used over many years in veterinary and human medicine, resistance is common and occurs across all groups of bacteria. The mechanisms of acquired resistance include: (i) energy-dependent efflux of antibiotic (membrane efflux proteins), or (ii) altered target whereby the ribosome is protected from binding of tetracyclines (Chopra et al., 1992). A third mechanism whereby the drug is attacked by enzymes liberated by the bacteria is possible. The genes mediating resistance may be carried on plasmids or transposons. Resistance to one tetracycline will generally produce cross-resistance to the others in the group. One of the exceptions is for *Staphylococcus* spp., which is discussed in Section Antimicrobial Spectrum and Clinical Uses. The newest tetracycline, tigecycline, is active against many organisms (e.g., methicillin-resistant staphylococci) that are resistant to older tetracyclines (Agwuh and MacGowan, 2006).

Table 34.1 Chemical and physical properties of tetracyclines

Drug	Molecular weight	pK_a
Chlortetracycline	478.88	3.3, 7.4, 9.3
Doxycycline	462.46	7.75
Minocycline	457.48	8.25
Oxytetracycline	460.44	7.75
Tetracycline	444.43	8.3, 10.2

Susceptibility testing: For susceptibility testing, tetracycline may be used to test all others in the class (CLSI, 2013; CLSI, 2015). Specific breakpoints are also available for minocycline and doxycycline in dogs, and doxycycline in horses (Table 34.2). For veterinary isolates, the

Table 34.2 Tetracycline breakpoints for susceptibility testing. Source: CLSI, 2015.

Drug	Bacteria	Animal	S (µg/ml)	I (µg/ml)	R (µg/ml)
Tetracycline[a]	*Mannheimia haemolytica* *Pasteurella multocida* *Histophilus somni*	Cattle	≤ 2	4	≥ 8
Tetracycline[a]	*Actinobacillus pleuropneumoniae* *Pasteurella multocida* *Streptococcus suis*	Pigs	≤ 0.5	1	≥ 2
Tetracycline	*Staphylococcus pseudintermedius*	Dog	≤ 0.25	0.5	≥ 1
Doxycycline	*Staphylococcus pseudintermedius*	Dog	≤ 0.12	0.25	≥ 0.5
Doxycycline	*Staphylococcus aureus, Streptococcus equi ssp. zooepidemicus, Streptococcus equi ssp. equi, Escherichia coli*	Horse	≤ 0.12	0.25	≥ 0.5
Minocycline	*Staphylococcus pseudintermedius*	Dog	≤ 0.5	1	≥ 2
Tetracycline	Enterobacteriaceae	Human	≤ 4	8	≥ 16
Tetracycline	*Staphylococcus, Streptococcus*	Human	≤ 2	4	≥ 8

S, susceptible; I, intermediate; R, resistant.

[a] The results of tetracycline susceptibility tests are used to predict susceptibility for chlortetracycline and oxytetracycline. Isolates susceptible to tetracycline are susceptible to minocycline and doxycycline.

susceptible breakpoint varies among species and is lower than the human breakpoints, which should be considered when interpreting susceptibility tests. The breakpoint for susceptible organisms in humans is ≤4 µg/ml for all organisms, except streptococci, which is ≤2 µg/ml.

Pharmacokinetic–Pharmacodynamic Properties

Based on an evaluation of tetracycline pharmacokinetic–pharmacodynamic properties (PK-PD), the effectiveness is best expressed as a ratio of the area-under-the-curve for a 24-hour interval to the minimum inhibitory concentration (AUC_{24}/MIC) (Agwuh and MacGowan, 2006). The optimal AUC/MIC ratio is in the range of 25–40, with Andes and Craig (2007) suggesting a value of 25. In veterinary studies this has been explored insufficiently. One study (Prats et al., 2005) examined PK-PD parameters after administration of doxycycline to swine in drinking water at 10 mg/kg. They reported high AUC/MIC ratios ranging from 60 for *Pasteurella,* 155 for *Mycoplasma,* and 585 for *Bordetella*. For *Actinobacillus,* which exhibits a higher MIC, the AUC/MIC was only 13. However, these ratios did not factor the protein binding, which is at least 90% (Table 34.3). Using a fraction unbound (*fu*) value of 0.1, reduces these ratios substantially. Ideal AUC/MIC ratios from their study would be achieved only for *Bordetella bronchiseptica*.

For *Staphylococcus* isolates from dogs, substantial PK-PD analysis was performed for doxycycline and minocycline (Maaland et al., 2013, 2014; Hnot et al., 2015a). It was concluded that AUC/MIC was the parameter that should be used for analysis, and a AUC/MIC ratio of 25 of the *unbound fraction* was optimum for predicting susceptibility and deriving dosages administered.

Table 34.3 Protein binding of tetracyclines in various species (references provided in text)

Drug	Species	% Protein binding
Chlortetracycline	Cows	47–51
	Sheep	46–50
Doxycycline	Calves	92
	Cats	98
	Horses	82
	Dogs	91–92
	Pigs	93
	Sheep	84–90
	Turkeys	70–85
Oxytetracycline	Buffalo	42
	Cows	18–22
	Horses	50
	Pigs	75.5
	Sheep	21–25
	Trout	55
Tetracycline	Cows	31–41
	Sheep	28–32
Minocycline	Dog	65.8
	Sheep	80

Absorption

Tetracyclines can be administered intravenously (most tetracyclines) or intramuscularly (oxytetracycline). The oral route also has been used (Table 34.4). Although some formulations (e.g., chlortetracycline and tetracycline) have been administered in feed and water for pigs, cattle, and poultry, the systemic effects of this route of administration may be much less than anticipated. In a study in which oxytetracycline was fed to pigs (Hall et al., 1989) the authors concluded that feeding this medication at a rate of 0.55 g/kg of feed resulted in plasma concentrations so low that only highly susceptible

Table 34.4 Comparison of oral absorption of tetracyclines in animals (mean values of studies reported; references listed in text)

Drug	Species	Systemic absorption (F%)
Chlortetracycline	Chickens	1
	Turkeys	6
	Pigs	6, 11, 19 (depending on the study and fasting conditions)
Oxytetracycline	Pigs	3–5
	Fish	6
	Turkeys	9–48
Tetracycline	Pigs	5, 8, 18, 23 (depending on the study and fasting conditions)
	Dogs	40
	Cats	50
Doxycycline	Pigs	21.2
	Chickens	41.3
	Turkeys	25, 37, 41, 63.5 (depending on age)
	Horses	17.3 (intragastric); 6 as a top dressing; higher in foals
	Dogs	53 (hyclate), 33.5 (monohydrate)
	Dogs	61.85 (summary of 4 studies)
	Calves	70
Minocycline	Dogs	50.3
	Cats	62

bacteria would be inhibited, with plasma concentrations reaching only one-tenth of the MIC for most pathogens. Several studies have examined use of tetracyclines added in subtherapeutic concentrations to feed rations (in particular, chlortetracycline) (Zinn, 1993; Jones et al., 1983; Dawson et al., 1983; Williams et al., 1978; Quarles et al., 1977; Richey et al., 1977; Nivas et al., 1976). The explanation for low oral absorption is not clear, but may be multifactorial. Tetracyclines are zwitterions and ionized at physiological pH values (see Table 35.1). Although the tetracyclines are relatively lipophilic drugs, they are ionized in the gastrointestinal tract and may not cross membranes easily. The oral absorption of tetracycline can be reduced in the presence of food (Nielsen and Gyrd-Hansen, 1996; Hnot et al., 2015b). Tetracyclines can easily chelate to polyvalent cations, which decreases the absorption several-fold. Thus, tetracycline oral absorption can be decreased with the coadministration of food, dairy products, polyvalent cations (i.e., Ca^{2+}, Mg^{2+}, Fe^{2+}, Al^{3+}), kaolin/pectin preparations, iron-containing supplements, and antacids (Weinberg, 1957; Waisbren and Hueckel, 1950; Harcourt and Hamburger, 1957; Neuvonen et al., 1970; Hägermark and Hoglund, 1974; Gothoni et al., 1972; KuKanich et al., 2014; KuKanich and KuKanich, 2015). When doxycycline or minocycline were administered with sucralfate (containing aluminum) oral absorption was significantly reduced unless the sucralfate was administered 2 hours after the tetracycline (KuKanich et al., 2014; KuKanich and KuKanich, 2015).

Distribution

Once absorbed, tetracyclines bind to plasma proteins to varying degrees in each species with doxycycline being greater than 80% in most animals (Riond and Riviere, 1989b) (Table 34.3). The high protein binding for some drugs may limit the microbiologically active fraction in the plasma. For the agents that are not restricted by protein binding, tetracyclines are widely distributed throughout most tissues of the body, including intracellular sites. Tables 34.5–34.9 show the pharmacokinetic variables for some of these drugs. High protein binding can limit tissue distribution. Bidgood and Papich (2003) showed that plasma protein binding for doxycycline was high (91.75%), which drastically limited the distribution into the tissue fluid. The same property was shown with doxycycline in horses (Davis et al., 2006). The plasma protein binding of 82% in horses reduced distribution to tissue fluid. Maaland et al. (2014) showed that plasma protein binding of minocycline limited the distribution to approximately 50% of the dose administered. Protein binding has less of an effect on distribution to intracellular sites and joint fluid (see below in this section).

Tetracyclines are moderately lipophilic (depending on the pH) compared to some other classes of antibiotics (e.g., β-lactams, and aminoglycosides); therefore, for the fraction not restricted by protein binding, at physiological pH they are capable of crossing lipid membranes. Some tetracyclines penetrate tissues better than others.

Table 34.5 Pharmacokinetic parameters of chlortetracycline in some food-animal species

Species	Dose (mg/kg)	Route	Vd (l/kg)	$t_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Turkey	0.9	IV	0.2284	0.877	3.77	Dyer, 1989
Pigs	11.0	IV	1.3883	NR	0.3071	Kilroy et al., 1990
Pigs	10	IV	0.7	3.6 (MRT)	3.33	Nielsen and Gyrd-Hansen, 1996
Pigs	39.9	Oral	—	8.7 (MRT)	—	Nielsen and Gyrd-Hansen, 1996
Calves (milk fed)	11.0	IV	3.34	8.89	260.52 l/h/kg	Bradley et al., 1982
Calves (conventionally fed)	11.0	IV	1.93	8.25	162.12 l/h/kg	Bradley et al., 1982

NR, information not reported; IV, intravenous; MRT, mean residence time.

Table 34.6 Pharmacokinetic parameters of tetracycline in some species

Species	Dose (mg/kg)	Route	Vd (l/kg)	$t_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Gilts	11	IA	1.06	NR	0.4	Kniffen et al., 1989
Chickens	65	IV	0.174	2.772	1.632	Anadon et al., 1985
Rabbits (male and female)	10	IV	1.047	2	6.1	Percy and Black, 1988
Channel catfish (*Ictalurus punctatus*) (27°C)	4	IV	0.513	16.5	0.365	Plakas et al., 1988
Pigs	11	IV	4.5	16	3.1	Kniffen et al., 1989
Pigs	9.6	IV	1.2	5.6 (MRT)	3.5	Nielsen and Gyrd-Hansen, 1996
Pigs	46.4	Oral	—	9.0 (MRT)	—	Nielsen and Gyrd-Hansen, 1996

NR, information not reported; IV, intravenous; IA, intraarterial; MRT, mean residence time.

Table 34.7 Pharmacokinetic parameters of oxytetracycline in some species

Species	Dose (mg/kg)	Route	Vd (l/kg)	$T_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Horses	10	IV	0.6728	12.953	0.6583	Horspool and McKellar, 1990
Ponies	10	IV	1.0482	14.949	1.013	Horspool and McKellar, 1990
Donkeys	10	IV	0.7765	6.464	1.523	Horspool and McKellar, 1990
Horses (adult)	2.5	IV	1.35	10.5	NR	Pilloud, 1973
Pigs	10	IV	1.49	5.99	2.88	Pijpers et al., 1991
Pigs (normal)	50	PO	1.44	5.92		Pijpers et al., 1991
Pigs (pneumonia)	50	PO	1.9	14.1		Pijpers et al., 1991
Pigs	20	IV	5.18	3.68	4.15	Mevius et al., 1986b
Cows (adult)	2.5	IV	1.04	9.12	NR	Pilloud, 1973
Dairy cows	5	IV	0.917	2.63	1.24	Nouws et al., 1985a, 1985b
Dairy cows[a]	5.23	IV	1.01	2.58	1.45	Nouws et al., 1985a, 1985b
Veal calves	40	IV	18.144	7.34	2.246	Meijer et al., 1993a
Veal calves	20	IV	18.541		2.167	Meijer et al., 1993a
Calves (3 weeks old)	7.54	IV	2.48	13.5		Nouws et al., 1983
Calves (12 weeks old)	6.88	IV	1.52	8.8		Nouws et al., 1983
Calves (14 weeks old)	17	IV	1.83	10.8		Nouws et al., 1983
Buffalo calves (female)	22	IV	0.32	3.6	1.02	Varma and Paul, 1983
Dogs	5	IV	2.096	6.02	4.23	Baggot et al., 1977
Rabbits	10	IV	0.668	1.32	14.6	McElroy et al., 1987
Turkeys	1	IV	3.622	0.7298	3.6579	Dyer, 1989
Rainbow trout	5	IV	2.988	81.5	0.423	Black et al., 1991
African catfish	60	IV	1.33	80.3	0.19	Grondel et al., 1989
Red-necked wallaby	40	IV	2.041	11.4	NR	Kirkwood et al., 1988
Foal	59	IV	1.95–2.2	6.7–7.3	3.3	Papich et al., 1995
Cattle	20	IM	3.34	21.6	—	Craigmill et al., 2004 (meta-analysis)
Cattle (young)	20	IV	0.94	5.67	—	Toutain and Raynaud, 1983
Calves	40	IM	—	23.9	—	TerHune and Upson, 1989
Calves (healthy)	11	IV	2.32	11.8	3.35	Ames et al., 1983
Calves (disease)	11	IV	3.6	14.8	4.01	Ames et al., 1983
Pigs	9.5	IV	1.4	6.5 (MRT)	3.67	Nielsen and Gyrd-Hansen, 1996
Pigs	45.5	Oral	—	10.3 (MRT)	—	Nielsen and Gyrd-Hansen, 1996
Sea turtles	25	IV	18.4	66.1	4.8	Harms et al., 2004
Sea turtles	25	IM	28.5 (Vd/F)	61.9	5.3 (CL/F)	Harms et al., 2004
Alligator	10	IV	0.77	74.1	0.12	Helmick et al., 2004

NR, or blank space, information not reported; IV, intravenous; PO, per os; MRT, mean residence time.
All formulations were reported to be or are assumed to be HCl unless otherwise noted.
Vd, apparent volume of distribution (Vd/F for non-IV dose forms); $T_{1/2}$, elimination half-life, or terminal half-life (for multicompartment models); Clearance, systemic clearance (CL/F for non-IV dose forms).
[a] Oxytetracycline dihydrate formulation tested.

Table 34.8 Some pharamcokinetic parameters of doxycycline in some species

Species	Dose (mg/kg)	Route	C_{max} (µg/ml)	Vd (l/kg)	$T_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Pigs (9 weeks old)	20	IV		0.53	4.04	1.67	Riond and Riviere, 1989
Pigs	12	Oral		—	7.2	—	Prats et al., 2005
Pigs	10.5	IV		0.89	4.2	2.8	Baert et al., 2000
Pigs	10.5	Oral		0.97	2.9	2.9	Baert et al., 2000
Horses	20	Oral	0.91	—	11.8	—	Davis et al., 2006
Horses	10	Oral	2.54	–	8.5	–	Womble et al., 2007
Horses	20	Oral	2.89	–	11.9	–	Womble et al., 2007
Horses	10	Oral	0.48	–	13.8	–	Winther et al., 2011
Horses	5	Oral	0.37	–	15.08	–	Schnabel et al., 2010
Calves	5	IV		–	9.5	1.2 (mg/l)	Meijer et al., 1993b
Calves (functional rumen)	20	IV		1.31	14.9	1.07	Riond et al., 1989
Calves (nonfunctional rumen)	20	IV		1.81	9.9	2.2	Riond et al., 1989
Cats	5	IV		0.34	4.56	1.09	Riond et al., 1990
Dogs	5	IV		0.93	6.99	1.72	Riond et al., 1990
Dogs	5	IV		1.468	10.36	1.68	Wilson et al., 1988
Dogs	0.1 CRI mg/kg/h	IV		0.65	4.56	1.66	Bidgood and Papich, 2003
Dogs	5–10	Oral	4.53	1.67	12.6	1.68	(summary of 3 studies)
Goats (lactating)	5	IV		9.78	16.63	6.91	Jha et al., 1989

CRI, constant rate infusion; IV, intravenous; NR, information not reported, C_{max}, peak concentration; Vd, apparent volume of distribution (Vd/F for oral dose); $T_{1/2}$, half-life (terminal half-life for multicompartment models). Clearance, systemic clearance (CL/F) for non-IV dose forms. All values listed are means from the study. Blank cells indicates that information was not available.

For example, minocycline and doxycycline, being more lipophilic (Barza et al., 1975), penetrate brain, ocular tissues, spinal fluid, and prostate better than other tetracyclines, such as oxytetracycline or chlortetracycline. Tetracyclines are commonly reported to concentrate intracellularly, and doxycycline has a higher affinity for intracellular accumulation than other tetracyclines (Gabler, 1991; Forsgren and Ballahsene, 1985; Davis et al., 2006). In vitro analysis of the penetration of radiolabeled doxycycline into isolated human polymorphonuclear leukocytes revealed a cellular-to-extracellular concentration ratio of 13 (Forsgren and Ballahsene, 1985). In horses the ratio was 17 at peak concentrations (Davis et al., 2006). These high leukocyte concentrations may contribute to the reported antiinflammatory effects. Minocycline is found in high concentrations in the bronchial secretions (Kelly and Kanegis, 1967a; MacCulloch et al., 1974); prostate (Fair, 1974); brain (Barza et al., 1975); thyroid, saliva, and tears (Hoeprich and Warshauer, 1974). Doxycycline is distributed to the epithelial lining fluid of the airways is high with 87% penetration after an intragastric dose. Distribution into joint fluid of horses has been shown, which has implications for both treating infections of the joint, and also for controlling joint inflammation (Schnabel et al., 2010, 2012; Maher et al., 2014). Distribution of

Table 34.9 Some pharmacokinetic parameters of minocycline HCl in some species

Species	Dose (mg/kg)	Route	C_{max} (µg/ml)	Vd (l/kg)	$T_{1/2}$ (hour)	Clearance (ml/min/kg)	Reference
Dogs (2-compartment model)	5	IV	NA	1.95	6.93	3.347	Wilson et al., 1985
Dogs (3-compartment model)	5	IV	NA	2.0	7.24	3.424	Wilson et al., 1985
Sheep (normal)	2.2	IV	NA	1.32	2.58	5.94	Wilson and Green, 1986
Sheep (hypoprotein-emic)	2.2	IV	NA	1.67	2.91	5.60	Wilson and Green, 1986
Cats	14 50 mg /cat	Oral	4.77	2.52	6.3	4.61	Tynan et al., 2015
Cats	5	IV	–	1.54	6.66	2.87	Tynan et al., 2015
Dogs	10	Oral	3.44	2.52	4.14	5.4	Maaland et al., 2014
Dogs	5	IV	–	1.46	6.02	2.85	Maaland et al., 2014
Dogs	6	Oral	3.44	2.17	5.8	4.27	Hnot et al., 2015a
Horse	4	Oral	0.67	14.95	11.5	12.03	Schnabel et al., 2012

IV, intravenous; NR, information not reported, C_{max}, peak concentration; Vd, apparent volume of distribution (Vd/F for oral dose); $T_{1/2}$, half-life (terminal half-life for multicompartment models).

Table 34.10 Formulations of tetracyclines used in animals

Formulations approved by US FDA for animals
Oxytetracycline hydrochloride soluble powder: added to drinking water for poultry, cattle, pigs
Oxytetracycline for medicated feed: added to feed for cattle, poultry, fish, pigs
Oxytetracycline tablets: oral treatment for calves
Oxytetracycline injection: IM injection for cattle and pigs. These products are occasionally used in horses and other species. There is both a conventional and long-acting formulation. The long-acting formulation contains a viscosity excipient used to prolong the absorption from the injection site
Tetracycline bolus: oral treatment for cattle
Tetracycline hydrochloride soluble powder: added to drinking water for cattle, pigs and poultry
Tetracycline oral suspension: oral treatment for cats and dogs
Chlortetracycline hydrochloride soluble powder: added to drinking water for poultry, calves, and pigs
Chlortetracycline for medicated feed: premix added to feed for pigs, cattle, poultry
Formulations approved for humans, but used off-label in animals
Doxycycline capsules and tablets: used in dogs, cats, birds, horses, and some exotic animals
Minocycline capsules, tablets, and injectable solution used in dogs, cats, and horses

doxycycline and minocycline to equine joint fluid was 4.6 times, and 2 times the plasma concentrations for doxycycline and minocycline, respectively.

Metabolism, excretion, and elimination

The elimination rates and half-lives are presented in the pharmacokinetic tables for each drug and various species (Tables 34.5–34.9). Although it varies considerably from species to species, the half-life is long enough for once or twice-daily administration in most animals. The intramuscular administration of formulations that contain viscosity excipients (e.g., 2-pyrrolidone) (Table 34.10) may prolong the terminal half-life because of a "flip-flop" effect. This is discussed in more detail in Section Oxytetracycline.

A high percent of the administered dose is eliminated in the urine via glomerular filtration, with the rest eliminated in the feces. The high concentrations in the urine can be effective for treatment of urinary tract infections caused by susceptible bacteria.

An examination of Tables 34.5–34.9 indicates that systemic clearance is similar to, or somewhat higher than GFR. Tetracyclines are also excreted in the bile, with up to 20 times the plasma concentration of tetracyclines being present in the bile (Kunin and Finland, 1961; Schach von Wittenau and Twomey, 1971).

Antimicrobial Spectrum and Clinical Uses

The use and dosages of specific agents in this group are discussed in this chapter for each drug. The value of the tetracyclines is their activity against susceptible gram-positive and gram-negative bacteria, as well as other atypical pathogens transmitted by ticks and other parasites, and blood-borne pathogens.

Tetracyclines in general have good or moderate activity against the respiratory pathogens listed in Table 34.2, but resistance can occur. Tetracyclines are usually active against *Bacillus* spp., *Corynebacterium* spp., *Erysipelothrix rhusiopathiae, Listeria monocytogenes,* streptococci, *Actinobacillus* spp., *Leptospira* spp., *Actinomyces* spp., and some anaerobes. The family Rickettsiaceae includes *Rickettsia* and *Ehrlichia,* and tetracyclines, particularly doxycycline, are considered the first drug of choice for these infections.

In birds, doxycycline is the drug of choice for treatment of *Chlamydophila psittaci* (formerly called *Chlamydia psittaci*). Tetracyclines are also useful against organisms that lack a cell wall, which would ordinarily be resistant to β-lactam antibiotics, for example, *Mycoplasma*, as well as other Mycoplasma organisms such as *Mycoplasma haemofelis* (formerly called *Haemobartonella felis*).

Resistance is common among *Enterococcus* species and members of the family Enterobacteriaceae (*Enterobacter* spp., *E. coli, Klebsiella* spp., *Proteus* spp., *Salmonella* spp.), and treatment should not be considered without a susceptibility test. Anaerobes (such as *Bacteroides* spp. and *Clostridium* spp.) have shown variable susceptibility. Commonly resistant to the tetracyclines are those infections involving *Mycobacterium* spp., *Proteus vulgaris, Pseudomonas aeruginosa,* and *Serratia* spp. Most *Streptococcus* spp. are susceptible, as are many *Staphylococcus* spp.; however, resistance can occur and a susceptibility test is advised before administering a tetracycline (e.g., doxycycline, minocycline) for treating staphylococcal infections in animals. Activity of minocycline against methicillin-resistant *Staphylococcus* has been shown. Staphylococci develop resistance through the efflux pump mediated by the gene *tetK.* These bacteria will be resistant to the other tetracyclines, including doxycycline, but not minocycline, which can still be used to treat some of these resistant infections (Hnot et al., 2015a; Weese et al., 2013; Maaland et al., 2014).

One of the clinical uses that has become common is administration of doxycycline in combination with other agents for treatment of canine heartworm disease. Tetracyclines, particularly doxycycline, are considered the first drug of choice for these infections. The *Rickettsia*-like organism found in heartworms, *Wolbachia*, is susceptible to tetracyclines, which have been used as adjunctive treatment for heartworm disease. Many filarial nematodes, such as heartworm, have a symbiotic relationship with obligate intracellular bacteria belonging to the genus *Wolbachia (Rickettsiales*). Treatment with doxycycline reduces *Wolbachia* numbers in all stages of heartworms and improves outcomes and decreased microfilaremia in dogs treated for heartworm disease. The American Heartworm Society recommends treatment with doxycycline in dogs diagnosed with heartworm disease. Preferably, it should be administered prior to treatment with an adulticide (melarsomine) at a dose of doxycycline of 10 mg/kg twice daily for 4 weeks. If doxycycline is not available, minocycline can be used as a substitute. In one assay, minocycline had better activity against *Wolbachia* than either doxycycline or rifampin (Townson et al., 2006).

Adverse Effects and Interactions

Interactions: Calcium-containing products or other di- or trivalent cations (Mg^{2+}, Fe^{2+}, Al^{+3}) will chelate with tetracyclines and interfere with oral absorption. Doxycycline is less susceptible to this interaction as calcium chelation is 19% for doxycycline but 40% and 36% for tetracycline and oxytetracycline, respectively (Barza et al., 1975). Interactions that affect oral absorption were discussed earlier in Section Absorption.

Gastrointestinal microflora changes: In horses the oral administration of oxytetracycline has been associated with proliferation of *Clostridium perfringens* or *Salmonella* in the colon, which has led to enteritis. This syndrome has been called *Colitis-X*. A more detailed discussion of the effects of tetracyclines in horses was reviewed by Papich (2003a, 2003b).

Esophageal lesions: Doxycycline entrapped in the esophagus from a broken tablet or incompletely dissolved capsule can cause injury to the esophagus and stricture. It has been demonstrated that, in cats, administration of a capsule or broken tablet can be lodged in the esophagus unless followed by some water. Therefore, one should be cautious about giving oral doxycycline medications to cats. This problem has been primarily associated with doxycycline hyclate (the form most common in the USA), rather than doxycycline monohydrate.

Problems in young animals: Tetracyclines bind to bone and teeth. They may produce teeth discoloration and inhibit growth of long bones in young animals or the offspring of pregnant animals treated with tetracyclines. The true incidence of this problem is not known in veterinary medicine, but in human medicine, tetracyclines are avoided in children less than 7 years of age (before tooth eruption). Tooth discoloration is determined by the duration of treatment rather than the dose. The discoloration is related to the chelation of tetracyclines to the calcium deposits in the developing teeth in the dentin (where it is mostly visible) and to a lesser extent in the enamel (Hamp, 1967; Hennon, 1965; Finerman and Milch, 1963; Moffitt et al., 1974). Exposure to sunlight is also important for this reaction. Although the occurrence of this in animals has not been well documented, it is prudent to avoid tetracyclines in animals during time of teeth development. The effects on bones are probably only important with high doses.

Renal tubular necrosis: Acute kidney injury has been associated with high doses and prolonged administration of oxytetracycline to ruminants (Riond and Riviere, 1989a) and dogs (Stevenson, 1980). When high doses are administered, the drug vehicle (such as propylene glycol) has been suspected to contribute to kidney effects.

Using outdated formulations: It often is stated in publications that kidney injury may occur when outdated tetracyclines are administered. The degradation products of the tetracyclines have been found to be nephrotoxic and are formed in the presence of heat, low pH, and moisture (Cleveland et al., 1965; Teuscher et al., 1982; Lowe and Tapp, 1966; Riond and Riviere, 1989a). Although we do not advocate administering outdated products, this problem does not occur with currently available formulations because the citric acid excipient is no longer used.

Hepatic disease: Idiosyncratic toxic hepatitis is possible (Böcker et al., 1982; Hopf et al., 1985). Drug-induced hepatitis has been described in people, and pregnant women appear to be at the greatest risk. The significance of hepatic reactions in veterinary medicine is unknown, but may be important at high doses.

Allergy: Hypersensitivity and drug fever have been reported. Cats appear to be more prone to drug fever from tetracyclines than other animals.

Photosensitivity: This is a direct toxic effect that damages cutaneous membranes when exposed to light. This reaction is rare in animals, but rather common in people. The incidence appears to be highest with doxycycline and demeclocycline.

Risks from IV administration: Tetracyclines administered intravenously rapidly can cause hypotension and collapse (McPherson et al., 1974; Wivagg et al., 1976; Gyrd-Hansen et al., 1981). In one study, a fast IV administration (60 seconds or less) to cattle caused collapse in 50% of the animals. Affected animals had low blood pressure, low heart rate, and ECG abnormalities. Collapse from IV injection has been prevented when the cattle were premedicated with calcium borogluconate, indicating that tetracycline may decrease the amount of

Table 34.11 Clinical dosages used for tetracyclines in animals – most frequently cited on product labels, CLSI susceptibility tables (CLSI, 2015), or in reputable references based on a consensus of the literature or pharmacokinetic studies

Drug	Species	Dose
Doxycycline	Dogs and cats	5 mg/kg q 12 h oral
Doxycycline	Horses	10–20 mg/kg q 12 h oral (never administer IV) The higher dose of 20 mg/kg will more consistently reach therapeutic targets
Oxytetracycline	Calves, cattle, and pigs	22 mg/kg q 24 h added to drinking water or in feed
Oxytetracycline	Calves, cattle	6.6–11 mg/kg q 24 h, IM
Oxytetracycline	Calves, cattle	20 mg/kg q 24 h, IM or SC; extra-label doses have been as high as 40 mg/kg
Oxytetracycline	Pigs	6.6–11 mg/kg q 24 h IM; doses as high as 20 mg/kg IM, q 24 h are also used
Oxytetracycline	Horses	10 mg/kg q 24 h, IM, or IV (slowly) (IM injections can cause pain)
Oxytetracycline	Dogs, cats	20 mg/kg, q 12 h, oral
Oxytetracycline	Sea turtles	40 mg/kg IM, followed by 20 mg/kg q 72 h, IM
Tetracycline HCL	Calves	11 mg/kg q 12 h PO
Minocycline	Horses	4 mg/kg q 12 h, PO
Minocycline	Dogs	5 mg/kg, oral, q 12 h (10 mg/kg can be considered for some organisms, but is more likely to produce vomiting)
Minocycline	Cats	8.8 mg/kg, oral, once daily (or 50 mg per cat, once daily)

calcium available to the heart for its role in contraction to the point of producing collapse of the animals.

The vehicle (solvent) used to administer tetracyclines may be responsible for adverse events. In calves, Gross et al. (1981) studied the cardiovascular effects of both oxytetracycline and the different vehicles used for injection (propylene glycol, saline, polyvinylpyrrolidine). They determined that the cardiovascular adverse effects were caused by the vehicles used and not oxytetracycline. The propylene glycol vehicle studied resulted in increased pulmonary arterial pressures and a decrease in cardiac output and stroke volume. Aortic pressure and heart rates were also depressed in association with the vehicle. They concluded that the cardiovascular effects observed were caused by endogenous release of histamine after propylene glycol injection, and this histamine release was not dependent on the animal being sensitized prior to exposure. No discernible cardiovascular effects were observed after injection with the oxytetracycline–saline combination, while the polyvinylpyrrolidine preparation and vehicle resulted in higher aortic pressure, heart rate, and overall systemic resistance.

Tetracycline has been reported to induce anaphylactic shock in dogs after intravenous injection (Ward et al., 1982) as well as possibly increasing alanine transaminase activity in the cat (Kaufman and Greene, 1993). Although there are warnings about administration of minocycline intravenously in humans, it has been administered to dogs and cats over a 5-minute interval without complication (Tynan et al., 2015; Maaland et al., 2014).

The most serious concern is intravenous injection of doxycycline in horses, which can be fatal (Riond et al., 1989a, 1992). IV administration of doxycycline to horses has caused sudden death, most likely caused by a cardiac arrhythmia. Oral administration of doxycycline to horses has not produced this problem (Davis et al., 2006).

Commonly Used Tetracyclines

Chlortetracycline

Chlortetracycline was the first tetracycline discovered and was first introduced for clinical use in 1948 (Figure 34.1). The use of chlortetracycline has mostly been confined to administration in feed and water to livestock. It has low oral absorption and the effects are likely caused by local effects in the intestine for weight gain. As mentioned earlier, production uses of tetracyclines for weight gain and improved feed efficiency have been phased out by an FDA guidance taking effect in 2017. Chlortetracycline is not utilized to any significant degree in small-animal or equine medicine. Doses for other animals are listed in Table 34.11. Because the therapeutic use has declined, the value of chlortetracycline has diminished. The reader is referred to earlier editions of this book for more detailed information about its past use.

Chlortetracycline has been used in pigs as a feed additive for the treatment of *Salmonella typhimurium* (Jones et al., 1983; Williams et al., 1978), coccidiosis (Onawunmi and Todd, 1976), and many other porcine diseases. Similar infections have been treated with chlortetracycline in poultry (Fagerberg et al., 1978; Nivas et al., 1976; Quarles et al., 1977; Landgraf et al., 1981; Dawson et al., 1983). Chlortetracycline has been reported to decrease the breeding rate of sows, although it did increase conception and farrowing rates. Birth weights, overall litter weights of pigs born alive, and weights of pigs at weaning were also significantly higher than unmedicated controls (Soma and Speer, 1975).

Tetracycline

The use of tetracycline (Figure 34.1) is more limited today because other forms are used in livestock (e.g., oxytetracycline) or in horses and small animals (minocycline, doxycycline). Relatively little has been published in recent years on tetracycline and previous editions of this book may be consulted for older information. Some pharmacokinetic information on tetracycline is available in Table 34.6.

There are still approved formulations of tetracycline for small animals. These forms are occasionally used to treat various diseases such as *Rickettsia rickettsii* (Rocky Mountain spotted fever) when doxycycline is not available. A study by Breitschwerdt et al. (1991) determined that tetracycline, chloramphenicol, and enrofloxacin were all equally effective in treating this disease in experimentally infected dogs. Tetracycline was also found to be efficacious in the treatment of canine ehrlichiosis (*E. canis*) (Amyx et al., 1971; Davidson et al., 1978). However, it was less effective at clearing ehrlichiosis in dogs compared to imidocarb dipropionate (Price and Dolan, 1980).

Oxytetracycline

The most commonly used tetracycline in food animals is oxytetracycline (Figure 34.1). The most complete pharmacokinetic analysis was performed by the Food Animal Residue Avoidance Databank (Craigmill et al., 2004) for oxytetracycline in cattle. This analysis was derived from 41 data sets and 25 published papers (489 data points). A metaanalysis of this data from a dose of 20 mg/kg IM of long-acting tetracycline yielded the following population data: half-life 21.6 hours, peak concentration (C_{max}) 5.61 μg/ml, clearance 0.115 l/kg/h, and volume of distribution per fraction absorbed (Vd/F) 3.34 l/kg. Other pharmacokinetic values are shown in Table 34.7. Doses are listed in Table 34.11.

The clinical usefulness of oxytetracycline has been documented in most domestic species of animals, and previous editions of this textbook should be consulted for historic work on oxytetracycline. Oxytetracycline has been a common treatment for lung infections associated with bovine respiratory disease (BRD). Although the in vitro susceptibility may not always be favorable (Table 34.2), oxytetracycline may accumulate in pneumonic lung preferentially over normal lung, and an increased volume of distribution has been shown in diseased animals, which may improve treatment outcome (Ames et al., 1983, 1985; Baxter and McKellar, 1990). Tissue levels are maintained for 24 hours after dosing. Other uses for cattle have included treating cases of *Moraxella bovis*/infectious bovine keratoconjunctivitis infections in calves (Smith and George, 1985; George and Smith, 1985; George et al., 1985, 1988), mastitis, and anaplasmosis. Although oxytetracycline has been used as an intrauterine infusion for cows with retained fetal membranes, this practice has been discouraged (Dinsmore et al., 1996). Intrauterine use in cows may not improve reproductive performance in cows with retained fetal membranes and may cause illegal residues in milk of dairy cows (Dinsmore et al., 1996; Stevens et al., 1995).

Oxytetracycline has been used to treat ehrlichiosis in dogs (Adawa et al., 1992) as an alternative to doxycycline. However, the use of oxytetracycline in small animals is limited because of a lack of convenient dose forms and greater use of doxycycline and minocycline.

In horses oxytetracycline is sometimes administered for treatment of Potomac horse fever (*Neorickettsia risticii*) (Palmer et al., 1992). Larson and Stowe (1981) reported high serum concentrations obtained in clinically normal horses given 10 mg/kg oxytetracycline intravenously, with serum concentrations peaking at 30 minutes postinjection (16.85 μg/ml) and high concentrations persisting through at least 240 minutes (4.67 μg/ml). Oxytetracycline penetrated well into pulmonary and renal tissue, as well as into bronchial fluid. In another study of oxytetracycline in horses, Brown et al. (1981) used a dose of 5 mg/kg intravenously and found a peak concentration of oxytetracycline in the serum at 0.5 hours after dose, with a steady decline in serum levels through 36 hours. Similar fluid concentration versus time profiles were also demonstrated for oxytetracycline detected in the synovial fluid, peritoneal fluid, and urine after intravenous injection, suggesting that oxytetracycline crosses those membranes easily and that the concentrations obtained would be adequate for combating such infections as *Corynebacterium equi, Streptococcus zooepidemicus,* and *Actinobacillus* spp., but with limited efficacy in treating some *Staphylococcus aureus, Escherichia coli,* and *Salmonella* spp., and no efficacy in treating common *Pseudomonas aeruginosa* pathogens.

The pharmacokinetic features of oxytetracycline for some species are shown in Table 34.7. Data on the pharmacokinetics of oxytetracycline is available for dogs (Baggot et al., 1977; Cooke et al., 1981), calves (Burrows et al., 1987; Banting et al., 1985; Banting and Baggot, 1996; Schifferli et al., 1982; Meijer et al., 1993a, 1993c; TerHune and Upson, 1989; Toutain and Raynaud, 1983), ponies and donkeys (Horspool and McKellar, 1990), horses (Larson and Stowe, 1981; Brown et al., 1981; Teske et al., 1973), foals (Papich et al., 1995), chickens (Black, 1977), swine (Nielsen and Gyrd-Hansen, 1996; Hall et al., 1989; Pijpers et al., 1990; Mevius et al., 1986b), sheep (Immelman and Dreyer, 1986), elephants

(Bush et al., 2000), fish (Black et al., 1991; Grondel et al., 1989), and other species (Teare et al., 1985; Martinsen et al., 1992; McElroy et al., 1987; Kirkwood et al., 1988). Oxytetracycline is relatively well behaved from a pharmacokinetic perspective, which allows easy extrapolation across species. A physiological based pharmacokinetic model was developed, which allowed extrapolation of oxytetracycline from dogs to humans after intravenous or oral administration (Lin et al., 2015).

Oxytetracycline is the most common injectable tetracycline for reptiles. The elimination of oxytetracycline from reptiles is slow, which allows for infrequent dose intervals. Harms et al. (2004) showed that the half-life in sea turtles was over 60 hours, which would allow for extended-interval dosing (41 mg/kg once IM, followed by 21 mg/kg every 72 hours IM). In another study, Helmick et al. (2004), showed that in alligators the half-life of oxytetracycline was 74 hours, which allows for long intervals between doses (for example every 5 days).

Oxytetracycline solution in propylene glycol is 50–100 mg/ml (Oxy-Mycin®, Terramycin, Oxy-Tet®), and is available as a solution in povidone (IM use only). A "long acting" preparation is available with the viscosity excipient 2-pyrrolidone (200 mg/ml) in formulations such as Liquamycin® LA-200. Absorption of oxytetracycline is known to vary with injection site in calves. A report by Nouws and Vree (1983) found that site-to-site intramuscular injection bioavailability varied widely at 52 hours postinjection, with bioavailability being 79% in the buttock, 86% in the neck, and 98% in the shoulder.

Administration of the long-acting formulation, particularly in food animals, is intended to prolong serum and tissue concentrations for long periods of time – usually every 48 hours, but may be up to 3–5 days for some pathogens. Several studies have described the pharmacokinetic patterns of the conventional and long-acting formulations in dogs, sheep, cattle, and pigs. Toutain and Raynaud (1983) examined the pharmacokinetic parameters of oxytetracycline with the 2-pyrrolidone carrier (long-acting formulation) injected intramuscularly in young beef cattle. This intramuscular formulation resulted in rapid development of serum concentrations of 4 μg/ml within 60–90 minutes, followed by persistence of these levels for approximately 12 hours. Serum half-life was calculated to be 21.8 hours, and bioavailability was 51.5%. Serum concentrations exceeding 0.5 μg/ml were found to persist for approximately 87 hours, in contrast to approximately 52 hours for the conventional formulation in another study using cattle (Mevius et al., 1986a). Davey et al. (1985) injected cattle with the conventional oxytetracycline hydrochloride or the long-acting formulation, both at a standard 20 mg/kg dose, and found that although the long-acting formulation had lower peak serum concentrations when compared to the conventional formulation, the long-acting formulation had a longer serum half-life (36.9 hours) than the conventional formulation (11.1 hours). In addition, the time it took for serum concentrations to drop below 0.5 μg/ml was 86.8 hours for the long-acting formulation and 51.5 hours for the conventional formulation. Similar findings have been reported for dairy cows (Nouws et al., 1985b), calves (Nouws and Vree, 1983), pigs (Nouws et al., 1990; Xia et al., 1983; Nouws, 1984; Banting and Baggot, 1996), dogs (Immelman and Dreyer, 1981), and sheep (Nouws et al., 1990).

Despite the advantages of the long-acting oxytetracycline formulation cited above, there are also studies that cast doubt on the value of a long-acting formulation. In one such study, the long-acting oxytetracycline (in 2-pyrrolidone) was compared to a conventional formulation in pigs at a dose of 20 mg/kg of each formulation (Hall et al., 1989). There was no difference in area-under-the-curve (AUC) or disappearance rate constant from either formulation. The authors concluded that the long-acting formulation did not provide an advantage for pigs.

Doxycycline

The most popular drug in this class for small animals and birds is doxycycline (Figure 34.1). It is available in two forms, doxycycline hyclate and doxycycline monohydrate. Doxycycline hyclate (a dimer of two molecules) has been used more commonly but the monohydrate also is available. Doxycycline hyclate (Vibra-Tabs, and Vibramycin) is available in tablets and capsules. There is also a flavored doxycycline calcium suspension and monohydrate suspension for people. Doxycycline hyclate tablets (Ronaxan) is approved for dogs and cats in some countries, and doxycycline monohydrate (VibraVet) approved in other countries. There are no reported differences between these two formulations with respect to oral absorption, but the hyclate form is associated with more injury to the esophagus (see Section Adverse Effects and Interactions). Doxycycline hyclate (Vibramycin IV) also can be administered IV to patients (except horses) that cannot tolerate oral medications. The IV formulation is reconstituted before use and is stable for only 12 hours following reconstitution (72 hours in refrigerator, 8 weeks in the freezer).

Doxycycline and minocycline (discussed in Section Minocycline) differ from tetracycline, oxytetracycline, and chlortetracycline in that they are more lipophilic (five- to tenfold increase), resulting in higher tissue penetration, higher intracellular penetration, larger volumes of distribution, and better overall antimicrobial properties (Barza et al., 1975).

The pharmacokinetics of doxycycline has been studied in dogs and cats (Wilson et al., 1988; Riond et al., 1990; Bidgood and Papich, 2003), pigs (Riond and Riviere, 1990a, 1990b; Prats et al., 2005), calves (Meijer et al., 1993b; Riond et al., 1989b), goats (Jha et al., 1989), rhesus monkeys (Kelly et al., 1992), horses and foals (Davis et al., 2006; Papich et al., 1995; Winther et al., 2011), and birds (Flammer et al., 2001, 2003; Powers et al., 2000; Prus et al., 1992; Greth et al., 1993). Some of the pharmacokinetic data for doxycycline for commonly encountered species of animals are listed in Table 34.8. Oral absorption was reported for various species (Table 34.4) and shown to be higher than for other tetracyclines. Doses are listed in Table 34.11.

High intracellular drug concentrations produce good activity against intracellular pathogens. Doxycycline is the first drug of choice for treatment of tick-borne infections caused by *Ehrlichia canis* and *Rickettsia,* as well as *Mycoplasma haemofelis* (formerly called *Haemobartonella felis).* Efficacy of doxycycline for rickettsial disease in animals was demonstrated by Breitschwerdt et al. (1997, 1999). The most common dose for dogs and cats is 5 mg/kg q12 h orally (25 mg/cat q12 h). It also has been considered one of the treatments of choice, in addition to azithromycin, for treatment of infections caused by *Bartonella* (Kordick et al., 1997; Brunt et al., 2006), although the most appropriate drug for *Bartonella* is still unknown (Brunt et al., 2006). The role of doxycycline for treatment of canine heartworm disease was discussed in Section Antimicrobial Spectrum and Clinical Uses. The effectiveness is attributed to the activity against the symbiont *Wolbachia.*

Doxycycline has also been used for infections in other species, including respiratory tract disease and systemic colibacillosis in poultry (Migaki and Babcock, 1977; George et al., 1977) and anaplasmosis in splenectomized calves (Kutter and Simpson, 1978).

An important use of doxycycline is in birds. Doxycycline has become a treatment of choice for psittacosis caused by *Chlamydophila psittaci* (formerly called *Chlamydia psittaci*) in birds because of its good oral absorption, tolerance, and efficacy (Flammer et al., 2001, 2003; Powers et al., 2000). The oral route is preferred for doxycycline because IM injections cause pain and tissue irritation and did not maintain therapeutic concentrations. Oral doxycycline can be administered to pet birds by simply adding doxycycline hyclate to drinking water. When doxycycline hyclate was added to drinking water at concentrations of 0.28 mg/ml and 0.83 mg/ml (280 and 830 mg/l), plasma concentrations in treated birds were maintained high enough for susceptible organisms during a 45-day treatment (Powers et al., 2000). Another study confirmed that when added to drinking water at a concentration of 0.8 mg/ml (800 mg/l) it produced effective concentrations in psittacine birds for a treatment duration of 42 days (Flammer et al., 2001). Lower water concentrations of 400 mg/l also may produce effective concentrations in some birds.

Minocycline

The administration of minocycline (Figure 34.1) is considered when doxycycline is not available, or an alternative is needed. Like doxycycline, it is more lipophilic than other tetracyclines (Barza et al., 1975) and generally has good oral absorption. Pharmacokinetics have been studied in dogs, cats, and horses and presented in Table 34.9. Doses are listed in Table 34.11.

Minocycline has been well tolerated, but as the dose is increased in dogs from 5 mg/kg to 10 mg/kg, more vomiting is expected. Intravenous administration has been tolerated in cats and dogs if administered slowly over 5 minutes (Tynan et al., 2015; Maaland et al., 2014). A toxicological study performed by Noble et al. (1967) examined the use of minocycline in Beagles administered a daily dose of 5, 10, 20, or 40 mg/kg intravenously for 1 month. Adverse effects occurred only in the high dose groups. Minocycline produced erythema of the skin and mucous membranes, characterized by papules around the eyes, muzzle, ears, and abdomen; the intensity of these lesions was directly proportional to the dose administered. Decreases in red blood cell packed cell volumes, hemoglobin concentrations, and red cell counts were noted in dogs receiving 10 mg/kg or more of minocycline intravenously. Similar adverse effects were noted by Wilson et al. (1985). Other toxicological studies with minocycline have been performed in dogs, rats, mice, and monkeys (Benitz et al., 1967).

Tissue distribution studies in dogs were reported by Maaland et al. (2014). After oral administration distribution to tissue fluids is approximately 50% of the plasma drug concentration. After a dose of 4 mg/kg oral to horses there was good penetration to equine joints (Schnabel et al., 2012). Minocycline appears to be minimally metabolized (Wilson and Green, 1986), but metabolism data are not available for all species. Systemic clearance values in dogs and cats suggest that glomerular filtration plays an important role in elimination (Tynan et al., 2015; Maaland et al., 2014).

Other Nonantimicrobial Uses of Tetracyclines

Tetracyclines also have been used as immunomodulating drugs and antiinflammatory drugs. This use of tetracyclines has focused on treatment of osteoarthritis, vasculitis, and dermatitis.

Dermatology

A review is available of the uses of tetracyclines in dermatology by Tsankov et al. (2003). The action of tetracyclines appears to be via inhibition of inflammatory cell infiltration. Tetracyclines also may affect cyclooxygenase (COX-2) mediated prostaglandin (PGE-2) synthesis during inflammation. The antiinflammatory activity was reviewed by Suomalainen et al. (1992). The combination of tetracycline and niacinamide has been used in dogs for the treatment of discoid lupus erythematosus, pemphigus foliaceus, ulcerative dermatosis of Collies and Shetland Sheepdogs (vesicular cutaneous lupus erythematosus), lupoid onychodystrophy, and sterile pyogranulomatous disease (including sterile nodular panniculitis) (Auxilia et al., 2001; Rothstein et al., 1997; White et al., 1992). The exact mechanism to explain the efficacy of this combination is uncertain, but some inflammatory mechanisms are probably important. However, as an antipruritic treatment, this combination is not impressive (Beningo et al., 1999).

Alone, tetracyclines have been used for conditions in which an antiinflammatory mechanism may play a role (Suomalainen et al., 1992). Doxycycline was used in one study of plasmacytic pododermatitis in cats (Bettenay et al., 2001). Remission of signs occurred in 26% of cats.

Angular Limb Deformities in Foals

Another use of oxytetracycline has been the administration of high doses to newborn foals for the purpose of correcting angular limb deformities (Madison et al., 1994; Kasper et al., 1995). The doses have been as high as 50–70 mg/kg, IV, q 48 h. The explanation for this effect of oxytetracycline in horses may be explained by the relaxation of tendons. In rats, oxytetracycline is known to decrease viscoelastic properties of tail tendons in young animals (Wintz et al., 2012). The pharmacokinetics in foals at this dose and adverse effects were explored by Papich et al. (1995) and no adverse effects were reported.

Arthritis

Both in vivo and in vitro studies have documented antiinflammatory, chondroprotective, and antiarthritic effects of tetracyclines, particularly doxycycline and minocycline. These studies have been summarized (Schnabel et al., 2010, 2012; Maher et al., 2014; Haerdi-Landerer et al., 2007). The effects on arthritis may be caused by decreased inflammatory mediators, such as prostaglandins, and the reduced matrix metalloproteinases (MMP). In calves, the predominant effect was lower activity of MMP. In horses, these effects may be possible even after administration of a low dose that produces concentrations below the MIC of bacteria (Maher et al., 2014).

Yu et al. (1992) indicated that doxycycline administered prophylactically markedly reduced the severity of osteoarthritis in dogs with surgically induced transactions of the anterior cruciate ligament. Inhibition of classical lesions in that model was felt to be due to doxycycline's ability to inhibit (chelate) metalloproteases (collagenase, gelatinase, stromelysin) in the degenerating cartilage of the canine knee.

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35

Aminoglycoside Antibiotics

Mark G. Papich and Jim E. Riviere

Aminoglycoside antibiotics have been used in veterinary and human medicine for many years and have retained their importance for treating serious and routine infections. They are particularly valuable for treating infections caused by gram-negative bacilli, including bacteria that may be resistant to other agents. Their therapeutic importance derives from the rapid bactericidal effects, pharmacokinetics derived from a large variety of animal species, and relatively low rate of resistance. These advantages must be weighed against their potentially toxicity, requirement for administration by injection for systemic use, and high potential to produce chemical residues in food-producing animals.

Pharmacology of Aminoglycosides

General Properties

Aminoglycosides include the familiar drugs gentamicin, amikacin, kanamycin, and tobramycin. They also include less familiar drugs such as neomycin, dihydrostreptomycin, and paromomycin. Spectinomycin has been included with aminoglycosides in some textbooks, but we have instead included it with the miscellaneous antibiotics in Chapter 36. Aminoglycosides are a class of antimicrobial compounds produced from strains of *Streptomyces* spp. or *Micromonospora* spp. fungi. Those produced from *Streptomyces* are spelled with "mycin" and those produced from *Micromonospora* are spelled with "micin". Chemically, they are aminocyclitols: hydroxyl and amino or guanidine substituted cyclohexane with amino sugars joined by glycosidic linkages to one or more of the hydroxyl groups. These molecules have excellent solubility in water but poor lipid solubility, and are thermodynamically stable over a wide range of pH values and temperatures (Lancini and Parenti, 1982; Leitner and Price, 1982; Nagabhusban et al., 1982; Pechere and Dugal, 1979). They are large molecules with molecular weights ranging from 450 to 585. The aminoglycosides are basic polycations with pK_a values that range from 7.2 to 8.8 (Ziv and Sulman, 1974; Katzung, 1984; Prescott and Baggot, 1988).

The chemical structure of gentamicin is shown in Figure 35.1. A search for gentamicin reveals several products that have been identified (e.g., gentamicin C_1, C_2, C_{1A}, A_2, and A_3). The commercially available form contains a complex of gentamicin C_1, C_2, and C_{1A} as sulfate salts in a mixture. The proportion of each compound in a gentamicin complex can vary among commercial products. The other commonly used aminoglycosides are shown in Figure 35.2 Amikacin is a semisynthetic form synthesized from kanamycin to increase antimicrobial activity. The various mechanisms of nephrotoxicity (binding to proximal tubule brush-border vesicles and phospholipids, inhibition of mitochondrial function, etc.) may be associated with the number of free amino groups on the aminoglycoside molecule. In general, the most ionized aminoglycosides (i.e., neomycin, with six groups) are more toxic and show greater binding affinity than the least ionized aminoglycosides of the class (i.e., streptomycin, with three groups) (Bendirdjian et al., 1982; Cronin, 1979; Feldman et al., 1981; Humes et al., 1982; Just and Habermann, 1977; Kunin, 1970; Lipsky and Lietman, 1982; Luft and Evan, 1980a, 1980b; Weinberg et al., 1980). Other structural characteristics may account for differences in toxicity within groups of drugs with similar total ionization potentials (i.e., netilmicin, tobramycin, amikacin, and gentamicin, all with five ionizable groups).

Mechanism of Action

Aminoglycosides exert their antibacterial action by irreversibly binding to one or more receptor proteins on the 30S subunit of the bacterial ribosome and thereby interfering with several mechanisms in the mRNA translation process. These include disrupting an initiation complex between the mRNA and the 30S subunit, blocking further translation and thereby causing premature chain termination, or causing incorporation of an incorrect amino acid in the protein product. Although most antimicrobials that interfere with ribosomal protein synthesis are

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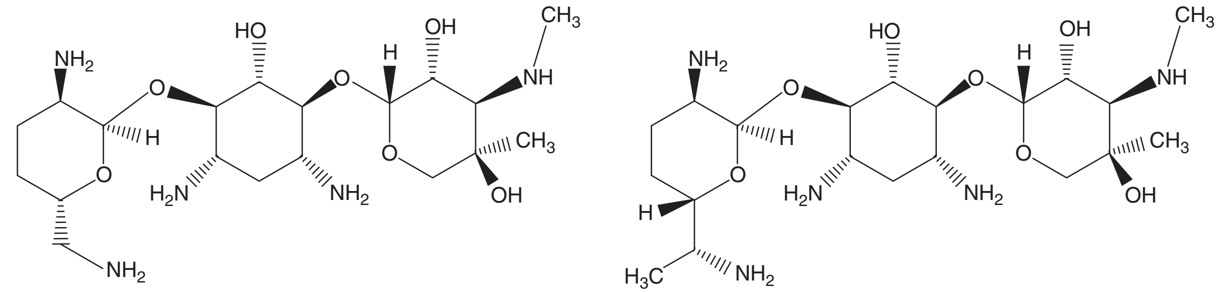
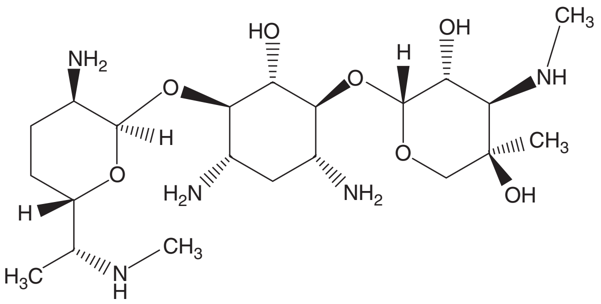


Gentamicin C_{1A}

Gentamicin C_2



Gentamicin C_1

Figure 35.1 Structures of gentamicin.

bacteriostatic, aminoglycosides are bactericidal. Because of irreversible binding, significant postantibiotic effects can be observed.

The mechanism of bacterial penetration by the aminoglycoside through the cell membrane is biphasic. Aminoglycosides diffuse through the outer membrane of gram-negative bacteria through aqueous channels formed by the porin proteins. Once in the periplasmic space, an oxygen-requiring process transports the drug into the cell, where it interacts with the ribosome. Anaerobic bacteria are therefore resistant to the antibacterial effects of aminoglycosides. The oxygen-dependent transport is linked to an electron transport system, which causes the bacterial cytoplasm to be negatively charged with respect to the periplasm and external environment.

An additional mechanism is independent of ribosomal binding. These agents are positively charged by virtue of their amino groups (Figures 35.1 and 35.2). These agents disrupt the cell surface biofilm, particularly on gram-negative bacteria, to produce disruption, loss of cell wall integrity, and a rapid bactericidal effect. Magnesium and calcium are important to cross-bridge adjacent lipopolysaccharide molecules. Aminoglycosides competitively displace Ca^{++} and Mg^{++} and destabilize the bacteria outer membrane. Therefore, rapid death of the bacteria can be caused by a cell surface effect rather than inhibition of the ribosome. This helps explain the concentration-dependent effect and rapid bactericidal action that is a feature of aminoglycosides. This property is not as prominent for gram-positive bacteria unless administered with a cell-wall disrupting agent such as vancomycin or a β-lactam antibiotic.

The positively charged aminoglycosides also affect the accumulation in bacteria. Because of the positive charge, they are attracted electrostatically into the bacterial cytoplasm. Some divalent cations (such as Ca^{++} and Mg^{++}) are competitive inhibitors of this transport system. This proton-motive force also functions in the lysosomes and mitochondria in which aminoglycosides accumulate and may also be a factor in the intralysosomal accumulation of the aminoglycosides.

A characteristic of aminoglycoside activity is that bacterial killing is concentration-dependent, and a postantibiotic effect (PAE) is evident. The PAE is a persistent suppression of bacterial growth following the removal of an antimicrobial agent. Bactericidal action persists after serum concentrations fall below minimum inhibitory concentrations (MICs). This has ramifications for the design of clinical dosage regimens.

Spectrum of Activity

Aminoglycosides are effective against most gram-negative bacteria, including gram-negative bacteria of the Enterobacteriaceae and *Pseudomonas aeruginosa*. They are effective against staphylococci, although

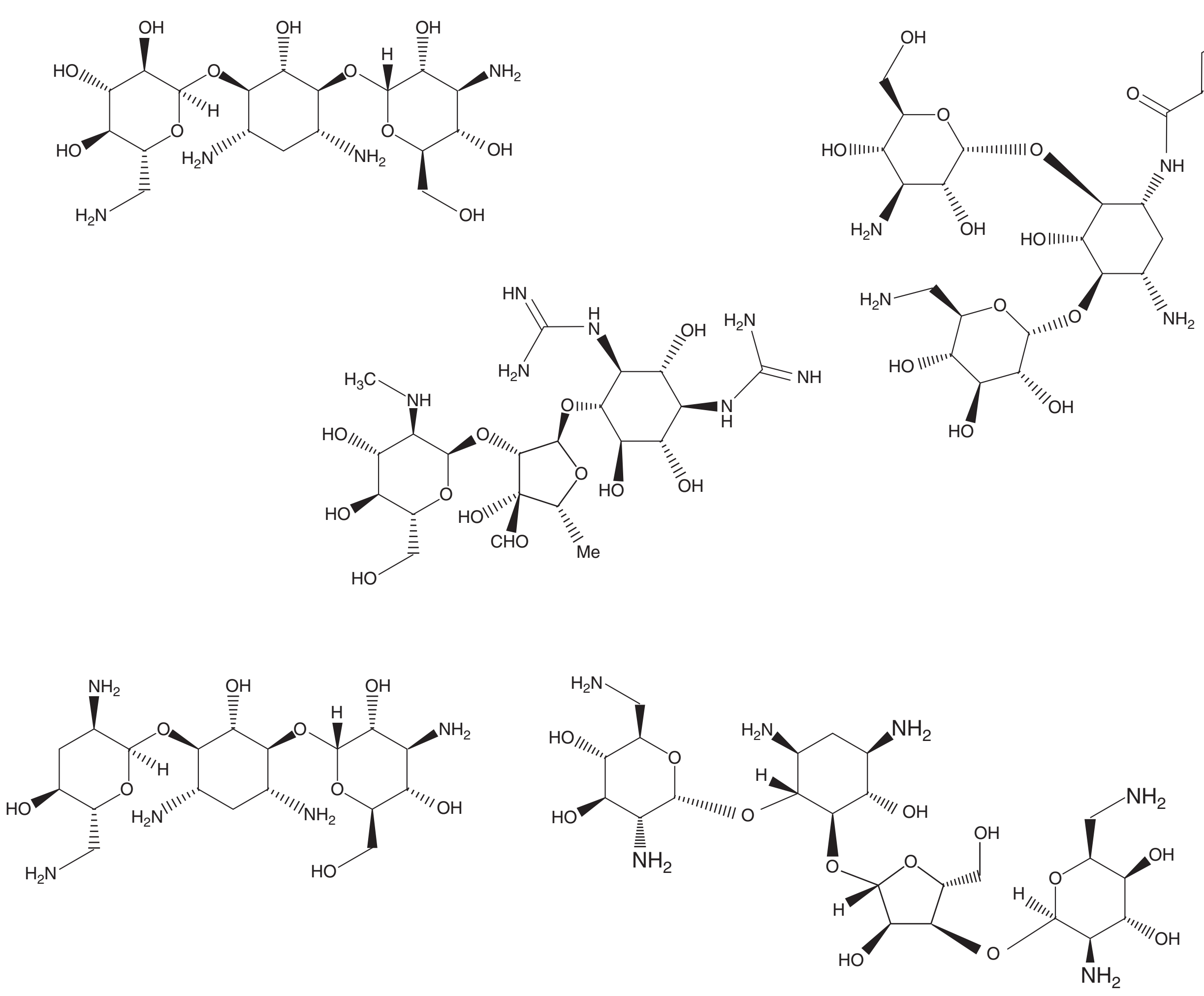


Figure 35.2 Structure of kanamycin, streptomycin, tobramycin, neomycin, and amikacin.

resistance can occur if used as monotherapy. Their action against streptococci and enterococci is limited unless they are combined with a β-lactam antibiotic. They have poor activity against *Pasteurella multocida*. Anaerobic bacteria are inherently resistant because drug transport into bacteria is oxygen dependent. Breakpoints for susceptibility testing have been established by the Clinical Laboratory Standards Institute (CLSI, 2015) and are shown in Table 35.1.

Comparison among drugs

Compared to other drugs in this group, amikacin usually has greater activity against gram-negative bacteria because it resists degradation by bacterial enzymes. This difference is observed with *E. coli* and particularly *Pseudomonas aeruginosa*. It is common for isolates obtained from dogs, cats, and horses to be resistant to gentamicin, yet still susceptible to amikacin. Gentamicin is approximately equal to tobramycin in activity, but tobramycin can be more active against some strains of *E. coli* and *Pseudomonas aeruginosa*. Kanamycin is least active compared to the others in this class, except for streptomycin.

Effect of tissue environment and other drugs on activity

pH effect: The action of aminoglycosides is pH dependent. The activity is less at low pH because high cation concentrations inhibit activity. The optimum pH for antibacterial activity is between 6 and 8. For example, gentamicin is 30 to 100-fold less active in an acidic (pH of 5.5 to 6.0) environment than at a pH of 7.4. Consequently, in some tissues and fluids (e.g., urine and abscesses) drug activity may be less because of lower pH.

Cellular debris: Aminoglycosides are bound to, and inactivated by, cellular debris and nucleic acid material that is released by decaying white blood cells. Therefore,

Table 35.1 Once-daily dosages for selected aminoglycosides

Species	Dose (in most cases the dose can be administered IV, IM, or SC)
Gentamicin	
Dog	9–14 mg/kg q 24 h
Cat	5–8 mg/kg q 24 h
Horse	Adult: 4–6.8 mg/kg q 24 h Foal (<2 weeks): 12–14 mg/kg q 24 h
Cattle	Adult: 5–6 mg/kg q24h Calf (<2 weeks): 12–15 mg/kg q 24 h
Sheep	Same as cattle
Amikacin	
Dog	15–30 mg/kg q 24 h
Cat	10–15 mg/kg q 24 h
Horse	Adult: 10 mg/kg q 24 h, IV, IM Foal (<weeks): 20–25 mg/kg q 24 h, IV

the activity in an abscess is poor. (One ml of purulent material can inactivate 700 μg of gentamicin.)

Oxygen tension: Low oxygen tension, such as that found in anaerobic tissue or decaying tissue, decreases the activity of aminoglycosides.

Cations: Because the uptake into bacteria is dependent on the drug's positive charge, divalent cations (e.g., Ca^{++}, Mg^{++}) can interfere with uptake of aminoglycosides into bacteria. Monovalent cations also may have some nonspecific inhibitory effect. The effects of cations on activity are discussed in Section Aminoglycoside Toxicity.

Other drugs: Aminoglycosides are inactivated if combined in vitro (for example in a vial or syringe) with other drugs, especially penicillins. This inactivation does not occur in vivo because concentrations in serum are not high enough to interact when two drugs are administered concurrently at the usual recommended doses. Aminoglycosides are synergistic with β-lactams against some bacteria in vitro, but this may not translate to improved clinical efficacy when the drugs are used simultaneously.

Resistance mechanisms

Anaerobic bacteria are intrinsically resistant because oxygen is necessary for aminoglycosides to enter bacteria. Resistance can occur by way of multiple effects. Some bacteria have an altered cell surface receptor, which is necessary to transport the drug into the bacteria. Bacteria can have a mutation in the target (ribosome) that resists binding, but this is uncommon.

A significant mechanism of resistance is degradation by bacterial enzymes. Several enzymes can be produced by bacteria that inactivate aminoglycosides. These enzymes can phosphorylate, adenylate, or acetylate groups on the molecule to render the drug inactive. The inactive drug can compete with the active drug for transport. Most drugs in this class are susceptible to many of the enzymes, but amikacin is susceptible to only one of the acetylase enzymes, which may account for amikacin's increased activity against some resistant bacterial strains in comparison to other aminoglycosides.

Pharmacokinetic–Pharmacodynamic Properties

The aminoglycosides are concentration-dependent bactericidal agents; therefore the higher the drug concentration, the greater the bactericidal effect. An optimal bactericidal effect occurs with peak drug concentration of 8–10 times the MIC, with little added benefit for concentrations above 10 times MIC. This target can be accomplished by administering a single dose once daily. This regimen is at least as effective, and perhaps less nephrotoxic, than lower doses administered more frequently (Freeman et al., 1997; Maglio et al., 2002; Drusano et al., 2007). Currently accepted dose regimens in small animals and horses employ this strategy. An additional benefit may be decreased resistance. According to Freeman et al. (1997), "Peak/MIC ratio of at least 10/1 may prevent the emergence of aminoglycoside-resistant pathogens". The single daily dose is usually calculated from the drug's volume of distribution. The peak may be achieved from IV, IM, or SC dosing. Total plasma concentration can be used because these drugs are essentially unbound (protein binding less than 10%).

Clinical Uses

The drugs used most often to any extent in veterinary medicine are amikacin, gentamicin, kanamycin, and neomycin (neomycin is used topically only). Netilmicin, sisomicin, and dibekacin are newer compounds but there are no reports of their use in veterinary medicine. Many streptomycin products have either been removed from the human market or are used only for certain infections (e.g., tuberculosis in people). Penicillin–dihydrostreptomycin combinations have been discontinued in the USA for use in animals.

Aminoglycosides are still considered to be important drugs of choice for treating serious aerobic gram-negative infections in veterinary medicine, although newer and less toxic antimicrobials (i.e., third-generation cephalosporins and fluoroquinolones) have replaced the use of aminoglycosides for some bacterial infections.

Neomycin is too toxic to be used systemically but is still used topically or oral for treating diarrhea. Kanamycin, was first introduced in the late 1950s, but many organisms are now resistant to this aminoglycoside and its use has subsequently declined. Gentamicin, introduced in the 1960s, has a broader spectrum and is associated with

Table 35.2 Susceptibility testing guidelines for aminoglycosides. Source: Data from CLSI. (2015). *Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria Isolated From Animals; Third Informational Supplement.* CLSI document VET01-S3. Wayne, PA: Clinical and Laboratory Standards Institute.

		MIC interpretive category (µg/ml)			
Drug	**Species**	**S**	**I**	**R**	**Comments**
Gentamicin	Dogs	≤ 2	4	≥ 8	Once-daily dose of 10 mg/kg
	Horses	≤ 2	4	≥ 8	Once-daily dose of 6.6 mg/kg
Amikacin	Dogs	≤ 4	8	≥ 16	Once-daily dose of 15 mg/kg
	Horses adult	≤ 4	8	≥ 16	Once-daily dose of 10 mg/kg
	Foals	≤ 2	4	≥ 8	For foals less than 11 days of age, 20 mg/kg, q 24 h, IV
Spectinomycin	Bovine	≤ 32	64	≥ 128	Respiratory pathogens

S, susceptible; I, intermediate; R, resistant.

less resistance than kanamycin. Amikacin, a semisynthetic derivative of kanamycin, was introduced clinically in the 1970s, has the broadest spectrum of activity of all the aminoglycoside antibiotics used clinically to date, and is the preferred antibiotic in severe gram-negative infections that are resistant to gentamicin or tobramycin.

Table 35.2 lists the dosage regimens for some of the aminoglycosides. It is important to note that these doses can be modified proportionately to correct for age, clinical or subclinical disease processes, renal insufficiency, or any of the other factors that may predispose the patient to aminoglycoside toxicosis (see Section Aminoglycoside Toxicity). Alterations in the dose can be best determined by monitoring serum creatinine concentrations or optimally by monitoring aminoglycoside serum concentrations at predetermined time points after dosing.

Single Daily Dose Administration

Because of the PK-PD properties, discussed in Section Pharmacokinetic–Pharmacodynamic Properties, single daily dosing of aminoglycosides may be as efficacious as administering the same dose divided over 24 hours. The concept of single daily dosing of aminoglycosides has been utilized and generally accepted within the human medical community (Bass et al., 1998; Christensen et al., 1997; Freeman et al., 1997; Karachalios et al., 1998; Rodvold et al., 1997). The efficacy of single dose administration is attributed to the rapid bactericidal action and the PAE, discussed in Section Mechanism of Action. Once-daily aminoglycoside dosage regimens that produce high peak and low trough concentrations also have less propensity to induce renal toxicity than multiple-dose regimens, which produce lower peak but higher trough concentrations. The clinical doses listed in Table 35.2 are derived from studies in these species that show that once-daily administration can achieve the targeted PK-PD value (Albarellos et al., 2004; Godber et al., 1995; Tudor et al., 1999; Martin et al., 1998; Magdesian et al., 1998; Bauquier et al., 2015; Tudor et al., 1999).

Local Administration

Intraarticular administration of aminoglycosides achieves higher concentrations in joint fluid compared to systemic therapy. This mode of administration may not be practical in all cases and is used most often in horses compared to other animals. In studies performed in experimental horses with septic arthritis, intraarticular administration of gentamicin (150 mg/joint) produced a much higher concentration in synovial fluid than IV administration. Twenty-four hours later, horses that received intraarticular gentamicin also had fewer bacteria in the synovial fluid. Amikacin and other drugs also have been administered via this route.

Aminoglycosides can be implanted directly using antibiotic-impregnated polymethylmethacrylate (AIPMMA) in the infection site. This material is a type of bone cement that hardens at the site once mixed and prepared. Antibiotics impregnated in this matrix results in high local concentrations that are released for a long period of time – sometimes for as long as 80 days. This technique avoids high systemic levels of drugs, reduces drug costs, and the need for frequent systemic administration. Aminoglycosides (tobramycin, amikacin, and gentamicin) are often used for this technique (Streppa et al., 2001).

Regional perfusion of antibiotics involves intravenous or interosseous administration of antibiotic in the limb of an animal while a tourniquet is applied proximal to the site of drug administration. This technique was reviewed by Rubio-Martinez and Cruz (2006). It has been performed in horses, cattle, and large zoo animals (e.g., elephants). It produces high concentrations of antibiotic in the distal limb (joint fluid and bone) of an animal (Murphey et al., 1999). High bactericidal concentrations are achieved during the interval when the tourniquet is applied. Then, concentrations quickly dissipate after tourniquet release. The high concentrations enter adjacent tissue via perfusion and can penetrate ischemic tissue and exudate via gradient diffusion. This technique reduces the total amount of drug used and maintains high concentrations in bone and joint fluid distal to the tourniquet. The advantage of regional limb perfusion has been that it confines the drug to the lower limb, preventing systemic exposure, and avoids the need for high systemic doses (Anderson et al., 1995). Drugs used in this technique have usually been amikacin, gentamicin, or tobramycin. For example, gentamicin has been used to treat infections in horses using regional limb perfusion, particularly for lower limb and joint infections (Whitehair et al., 1992a, 1992b).

Susceptibility Testing

Clinical breakpoints have been established by the Clinical and Laboratory Standards Institute (CLSI) through an analysis of pharmacokinetics, PK-PD criteria, and MIC distributions. These breakpoints are listed in Table 35.1.

Regulatory Status

Although they are not banned by the US FDA, much of the use in food-producing animals is considered extralabel, except for a few oral products used to treat diarrhea. Under the animal medical drug use clarification act (AMDUCA) of 1994, extralabel use is not allowed if there are other approved animal products effective for the condition being treated. More information is available in Chapters 52 and 61 of this book. These drugs have very long withdrawal times for slaughter. As long as 18 months for withdrawal prior to slaughter in cattle is suggested by the food animal residue avoidance data bank (FARAD). The American Association of Bovine Practitioners (AABP) and Academy of Veterinary Consultants (AVC) have recommended that, until further scientific information becomes available, aminoglycosides should not be used in cattle.

Pharmacokinetics of Aminoglycosides

General

A comprehensive review of aminoglycoside pharmacokinetics has been reported by Brown and Riviere (1991) and is found in earlier editions of this book. The pharmacokinetics of the aminoglycosides is similar across species lines, but the variability within each animal population is large, indicating a significant amount of heterogeneity in aminoglycoside disposition in both diseased and normal animals (Sojka and Brown, 1986; Frazier et al., 1988). Although there is variability in aminoglycoside pharmacokinetic parameters, the therapeutic range for all of the aminoglycosides is relatively narrow, and the potential for toxicosis is greater than for most other classes of antimicrobials. Altered physiological or pathological states such as pregnancy (Lelievre-Pegorier et al., 1985), obesity (Sketris et al., 1981), subnormal body weight (Tointon et al., 1987), kidney disease (Frazier and Riviere, 1987; Martin et al., 1998; Martin-Jimenez and Riviere, 2001), dehydration (LeCompte et al., 1981; Brown et al., 1985a), immaturity (Sojka and Brown, 1986), sepsis (Mann et al., 1987), dietary protein (Grauer et al., 1994; Behrend et al., 1994), endotoxemia (Wilson et al., 1984; Jernigan et al., 1988c), and intraindividual variability (Mann et al., 1987), among many others, may alter the distribution, clearance, and half-life of aminoglycosides by as much as 1000-fold between individuals in a single study (Zaske et al., 1982).

Absorption

Aminoglycosides are not appreciably absorbed from the gastrointestinal tract because of their highly polar and cationic nature. However, if there is significant disruption of the intestinal mucosa from enteritis (Gemer et al., 1983; Miranda et al., 1984; Gookin et al., 1999), some absorption may occur. For example, neomycin administered orally to calves with enteritis could increase the risk of residues at slaughter. The aminoglycosides are not inactivated in the intestine and are eliminated in the feces unchanged after oral administration to normal animals. This lack of significant absorption through the gastrointestinal tract requires that all aminoglycosides be given by parenteral routes if therapeutic plasma concentrations are desired. Aminoglycoside absorption is practically complete after IM or SC injection. The peak serum concentrations after extravascular injection occur 14–120 minutes after the dose (Blaser et al., 1983; Ristuccia, 1984). Absorption is extremely rapid and complete if aminoglycosides are instilled into body cavities that contain serosal surfaces; administration by this route closely mimics parenteral administration (Jawetz, 1984; Sande and Mandell, 1985). Absorption from topical administration in open wounds also is possible and may increase the risk of nephrotoxicosis if high doses are used (Mealey and Boothe, 1994).

Distribution

The aminoglycoside antibiotics are highly hydrophilic and distribute rapidly in extracellular body fluids. Because of their polycationic nature, the penetration of aminoglycosides across membranous barriers by simple diffusion is limited; therefore, low concentrations of aminoglycosides are found in cerebrospinal fluid or in respiratory secretions (Riviere and Coppoc, 1981b; Strausbaugh and Brinker, 1983). Aerosol or intratracheal administration of aminoglycosides produces negligible serum concentrations in animals and this has been used for in-hospital treatment of bronchitis. Through this route, substantial bronchial and pulmonary concentrations can be achieved (Riviere et al., 1981b; Wilson et al., 1981). Delivery with devices that nebulize these agents for delivery to the airways has been employed in hospitalized patients with gentamicin, amikacin, and tobramycin.

Plasma protein binding is negligible for all drugs in this group. These drugs easily pass from the capillaries through fenestrations in capillaries to achieve concentrations in interstitial fluids that are equivalent to plasma drug concentrations. The volume of distribution is approximately equal to the volume of extracellular fluid (typically in the range of 20–25% for most adult animals).

Physiological changes can alter the distribution. Decreases in body water (dehydration) can decrease the volume of distribution and increase plasma drug concentrations. Increases in body water caused by pregnancy, third-compartment fluid accumulation (e.g., ascites), and young age (neonate) will increase the volume of distribution and lower plasma drug concentrations. In studies performed in calves, foals, and puppies, the high body water – particularly extracellular water – produces a high volume of distribution for aminoglycosides. Because the plasma concentration is proportional to the volume of distribution: the larger the volume of distribution, the higher the dose that is needed to attain a targeted peak plasma concentration (C_{max}). For example, the volume of distribution for gentamicin or amikacin in foals is more than double the value for adult horses. Subsequently the dose needed to maintain the same blood concentration should be increased, at least by twofold.

Metabolism and Excretion

Several studies in animals (Black et al., 1963; Chiu et al., 1976; Chung et al., 1980; Gyselynck et al., 1971; Schentag and Jusko, 1977; Silverman and Mahon, 1979) have clearly demonstrated that aminoglycosides are eliminated nonmetabolized from the body in all animal primarily by renal glomerular filtration. Some degree of proximal tubular reabsorption occurs and results in an intracellular sequestration or storage in the tubule cells without a significant transepithelial flux from the intraluminal to peritubular space. Net aminoglycoside secretion along more distal nephron segments may also occur. Proximal tubule luminal absorption of aminoglycoside appears quantitatively to be the primary mechanism of intracellular uptake; however, selective peritubular or basolateral reabsorption, evident in isolated tissue slice studies, does occur and may be of toxicological significance in specific situations. Reabsorption requires metabolic energy and occurs along the midconvoluted and straight portions of the proximal tubule (Barza et al., 1980; Bennett et al., 1982; Hsu et al., 1977; Kaloyanides and Pastoriza-Munoz, 1980; Kluwe and Hook, 1978a, 1978b; Kuhar et al., 1979; Pastoriza-Munoz et al., 1979; Senckjian et al., 1981; Silverblatt, 1982; Silverblatt and Kuehn, 1979; Silverman and Mahon, 1979; Tulkens and Trouet, 1978; Vandewalle et al., 1981; Williams et al., 1981a, 1981b; Zaske, 1980). Renal cortical uptake of the aminoglycosides is dose-dependent up to a threshold concentration; then, cortical accumulation increases at a progressively slower rate as the dose is increased. Cumulative uptake of aminoglycosides in tissues indicates that the kidney is the major site of drug sequestration.

A typical plasma vs time profile for IV administration of an aminoglycoside antibiotic to animals shows three phases. The α (distribution) phase occurs within the first hour after IV dosing, the β phase (elimination) occurs between 1 and 24 hours after IV dosing (and probably the most useful in determining dose adjustments in clinical situations), and the γ phase occurs 24 hours after dosing and is the most important part of the elimination curve of aminoglycosides when considering drug residues in food-producing animals. Values for the beta- and gamma-phases are shown in Table 35.4 for gentamicin. The primary difference in pharmacokinetics among species is related to the glomerular filtration rate (GFR). The GFR is lower for larger animals because of allometric scaling; therefore, larger animals tend to have slower clearance and the half-lives are longer (Riviere, 1985; Riviere et al., 1997). Reptiles have lower GFR and lower renal clearance of aminoglycosides. This produces longer half-lives in reptile species.

The prolonged terminal elimination phase of aminoglycosides has major implication for veterinary therapeutics in food-producing animals. As discussed in Section Metabolism and Excretion, aminoglycosides accumulate in the renal cortex for prolonged periods of time, resulting in violative tissue residues even after short periods of administration. In some cases, aminoglycosides such as gentamicin may be detected for a year after parenteral administration! A withdrawal time of 18 months for cattle treated with gentamicin has been recommended by FARAD (see Chapter 61), but it is best to simply avoid use in these species altogether. Piglets may be treated up to 3 days of age with oral products, but even in this case the withdrawal time is 40 days.

Pharmacokinetics in Nonmammals

Veterinarians involved in nonmammal practice should be aware of variations in elimination in some animals. In birds the elimination half-life is usually 2–3 hours and dosing intervals are similar to what has been used in mammals. For amphibians and reptiles, however, elimination rates are much slower. Half-lives range from 38 to 72 hours in alligators and dose intervals of 72 to 96 hours have been used. In snakes half-lives can be as long as 80–121 hours. In turtles and tortoises, the half-life of

Table 35.3 Risk factors that predispose to aminoglycoside toxicosis

Age
Volume contraction (shock)
Acidosis
Sodium or potassium depletion
Sepsis
Renal transplantation
Prior renal insufficiency
Prior aminoglycoside exposure
Cumulative dose of aminoglycoside
Peak and trough serum concentrations
Hepatic disease
Total dose of drug administered
Duration of treatment
Concurrent administration of loop diuretics
Methoxyflurane anesthesia
Cephalosporin antibiotics
Nephrotoxic drugs

aminoglycosides has been in the range of 20–70 hours, with dose intervals usually every 48 hours to every 96 hours. Kidney injury may be greater because of the slower elimination in reptiles. Therefore, use these drugs cautiously in animals with slow clearance.

Aminoglycoside Toxicity

Aminoglycoside toxicity in domestic and laboratory animals was reviewed extensively by Riviere (1985). The possible risk factors that may predispose a patient to aminoglycoside toxicity are shown in Table 35.3.

Aminoglycosides can induce ototoxicity and nephrotoxicity because both organs have higher-than-normal concentrations of phospholipid (in particular, phosphatidylinositol) (Sastrasinh et al., 1982a, 1982b) in their cellular matrixes. Cationic aminoglycosides are chemically attracted to anionic membrane phospholipids. The tissues into which gentamicin preferentially accumulates (renal cortex and cochlear tissue) have disproportionately high amounts of phosphatidylinositol in their membranes compared with other tissues of the body (Hauser and Eichberg, 1973). Basolateral membranes of the renal proximal tubular epithelium also have a higher capacity for binding aminoglycosides than brush-border membranes because of their higher phosphatidylinositol content (Josepovitz et al., 1985).

Ototoxicity studies in a variety of species have shown injury from aminoglycosides that may affect both auditory and vestibular function due to destruction of the sensory hair cells in the cochlea and vestibular labyrinth. The mechanism of ototoxicity was described in a review (Lanvers-Kaminsky et al., 2017). Initially the outer hair cells of the cochlea are affected, which impars hearing at high frequencies. With continued exposure the inner hair cells are injured, which causes additional hearing impairment and deafness. Injury may be caused by oxidative stress and inhibition of mitochondrial protein synthesis. Aminoglycosides enter the inner ear by active transport mechanisms. Once in the ear, they are cleared slowly with half-lives of 10–13 days after a single dose, but up to 30 days after multiple doses. Ototoxicity may be irreversible in some cases (Johnson and Hardin, 1992). Of pertinence to veterinary medicine, dogs tend to present with auditory toxicity, and cats tend to present with vestibular toxicity, although both usually occur after nephrotoxicity has ensued.

The interaction between the cationic aminoglycosides and the kidney anionic phospholipids appears to be electrostatic and proportional to the cationic charge of the drug. This interaction is saturable and is competitively inhibited by divalent cations (magnesium and calcium), spermine, poly-L-lysine, and other aminoglycosides. For example, diets high in calcium, or calcium supplementation may decrease the risk of aminoglycoside nephrotoxicity (Schumacher et al., 1991; Brashier et al., 1998). After binding, the aminoglycoside is internalized into the cell by pinocytosis (Bennett et al., 1982; Elliott et al., 1982; Feldman et al., 1981; Humes et al., 1982; Lipsky et al., 1980; Lipsky and Lietman, 1982; Pastoriza-Munoz et al., 1979; Schacht, 1978), where concentrations of the aminoglycoside can reach as high as 50 times the concentrations achieved in serum or plasma. The uptake of aminoglycosides into lysosomes is competitive and is dependent in part upon the charge density of the aminoglycoside molecule, which is a function of the number of amino groups. For example, neomycin (valence + 4.37 at pH 7.40) accumulates in the renal cortex more than gentamicin (valence + 3.46 at pH 7.40) due to a higher cationic charge.

There are several mechanisms that may explain the mechanism by which aminoglycosides initially damage the proximal renal tubule cells (Swann et al., 1990; Schumacher et al., 1991; Beauchamp et al., 1992). Lysosomal dysfunction is a component of the early phase of renal injury (Carbon et al., 1978; Feldman et al., 1982; Hull et al., 1981; Kaloyanides and Pastoriza-Munoz, 1980; Laurent et al., 1982; Lipsky and Lietman, 1982; Mazze, 1981; Meisner, 1981; Morin et al., 1980, 1981; Tulkens and Trouet, 1978). This view is consistent with the idea that lysosomes are the primary locus of aminoglycoside sequestration in proximal tubule cells. Lysosomes are also the first organelle to demonstrate morphological changes (myeloid body or cytosegresome formation) after exposure to the drugs (Riviere et al., 1981a). Decreased lysosomal function may also result in a decreased ability to degrade endogenous intracellular proteins and exogenous low-molecular-weight proteins reabsorbed from the tubular filtrate, events that would perturb nephron function (Cojocel et al., 1983; Cojocel and Hook, 1983). The increase in lysosomal permeability could result in proximal tubule cell dysfunction, although this event is probably a late change in aminoglycoside-induced toxic nephropathy occurring after cell necrosis has been initiated by another factor (Humes et al., 1982). The appearance of lysosomal enzymes (for example, urinary γ-glutamyl transferase, GGT) in the urine of aminoglycoside-induced toxic nephropathy patients is secondary to proximal tubule cell necrosis, apical plasma membrane damage, or lysosome exocytosis.

Mitochondria are a second possible target of aminoglycosides because, both in vitro and in vivo, aminoglycosides decrease mitochondrial respiration, thereby impairing the tubule cell's bioenergetic profile (Appel and Neu, 1977; Cuppage et al., 1977; Kaloyanides and Pastoriza-Munoz, 1980; Kluwe and Hook, 1978a; Sastrasinh et al., 1982b; Simmons et al., 1980; Weinberg et al., 1980, 1990; Weinberg and Humes, 1980). This could selectively produce tubule dysfunction, which would initially be detectable biochemically but not morphologically. The mechanism of this toxicity may be secondary to a direct aminoglycoside interaction with mitochondrial membrane phospholipids, to a competitive interaction with the divalent cations magnesium or calcium, or to an alteration in the intracellular milieu that would indirectly affect mitochondrial function. The magnitude of aminoglycoside effects on mitochondrial respiration is associated with the net positive charge of the specific drug.

The third possible site of initial intracellular aminoglycoside is an interaction with the proximal tubule cell plasma membrane's phospholipids and enzymes (Feldman et al., 1981; Humes et al., 1982; Knauss et al., 1983; Lullmann and Vollmer, 1982; Sastrasinh et al., 1982a, 1982b; Schacht, 1979; Silverman and Mahon, 1979; Williams et al., 1981a, 1981b). Binding of aminoglycosides to membrane polyphosphoinositides could perturb the regulation of membrane permeability, thereby promoting cellular dysfunction. The enzyme interactions at the basolateral membrane could result in significant cellular dysfunction by altering intracellular electrolyte balance or osmolality.

An additional site of aminoglycoside interaction with the nephron is at the level of the glomerulus, where gentamicin has been demonstrated to reduce the glomerular ultrafiltration coefficient and to reduce the number and size of glomerular endothelial fenestrae (Avasthi et al., 1981; Huang et al., 1979; Luft and Evan, 1980a, 1980b; Luft et al., 1978). These effects may be mediated by a charge interaction between the cationic aminoglycosides and the anionic endothelial cell surfaces or could be a feedback response to a primary tubular injury (known as tubuloglomerular feedback).

The relative contributions of the lysosomal, mitochondrial, and membrane tubular mechanisms and glomerular injury to clinical aminoglycoside-induced toxic nephropathy is not known. It is possible that cellular dysfunction is a result of a combination of the above processes.

Dogs

Aminoglycoside-induced kidney injury in dogs follows a progression that consists of an initial subclinical (subazotemic) phase marked by a urinary concentrating defect followed by a clinical (azotemic) phase. It also serves as the basis for simple noninvasive clinical monitoring (for example, monitoring urine specific gravity and proteinuria) for toxicosis since urinary changes preceded the more irreversible systemic changes. If identified early, aminoglycoside-induced kidney injury can recover.

Urine GGT (γ-glutamyl transferase):creatinine and NAG (*N*-acetyl-β-D-glucosaminidase):creatinine ratios and 24-hour urinary excretions of NAG and GGT have been used as markers of aminoglycoside-induced kidney injury. Elevated GGT:creatinine ratio precedes clinically significant elevations in serum creatinine, urine specific gravity, and urine protein:creatinine ratios.

Risk factors in dogs (Brown et al., 1985a) were identified that contributed to nephrotoxicosis in 10 dogs. Risk factors included dehydration, fever, old age, and preexisting renal disease. In addition, low protein and electrolyte abnormalities were documented in these dogs. Other risk factors are shown in Table 35.3.

Ototoxicity in dogs, manifested as either vestibulotoxic and/or ototoxic effects, can occur after systemic aminoglycoside therapy, but toxicity after topical use of aminoglycosides is apparently rare (Strain et al., 1995). Although it is sometimes recommended among dermatologists to avoid topical gentamicin in animals with a ruptured tympanum (ear drum) this apparently is not a risk. In a study designed to detect ototoxicity in dogs treated with topically administered gentamicin using brain stem auditory evoked potential (BAEP), dogs underwent a unilateral myringotomy, followed by instillation of 7 drops of the 3 mg/ml buffered aqueous solution of gentamicin instilled into one ear twice a day for 3 weeks. There was no evidence in any treated dogs of drug-induced detectable changes in cochlear or vestibular function.

Cats

Cats have a relatively more concentrated urine and retain the ability to produce concentrated urine even when the GFR is significantly reduced (Ross and Finco, 1981), making urine monitoring less successful than in dogs. Consistent with the studies cited in dogs, studies in cats have shown that high doses, prolonged administration, or both, can produce kidney injury, with histological changes and increases in serum urea nitrogen and creatinine (Welles et al., 1973; Waitz et al., 1971).

Nephrotoxicosis associated with the topical use of gentamicin has been reported in cats (Mealey and Boothe, 1994). A cat was administered 10 ml of an undiluted gentamicin injectable solution (50 mg/ml) to lavage an open wound twice. The cat eventually progressed to an azotemic state and was euthanized. Histologically, the kidneys showed severe acute proximal tubular necrosis compatible with aminoglycoside toxicosis. Elevated serum levels of gentamicin were noted as late as 96 hours after administration. Although a number of factors may have contributed to the death of this cat, the topical administration of such large quantities of gentamicin was most likely the major determinant.

Horses

As in other animals, aminoglycoside-induced kidney and otic injury has been documented in horses (Nostrandt et al., 1991). Clinically, aminoglycoside-induced toxic nephropathy is more common in young animals, with toxicity rarely reported in adults (Riviere et al., 1982; Tobin, 1979). As in other animals the injury is marked by elevations in serum creatinine and serum urea nitrogen (Tobin, 1979; Riviere, 1982). The shift in dosing regimens from multiple times per day, to once per day has apparently decreased the risk of aminoglycoside-induced kidney injury in recent years and is now the accepted protocol used clinically (Tudor et al., 1999; Geor and Papich, 2003; Godber et al., 1995).

Examples of Drugs

Gentamicin

Gentamicin is available in solutions of 5, 50, and 100 mg/ml (Garasol, Gentocin, and generic), as well as oral solution for pigs (4.35 or 5 mg/ml) and powder for oral solution (66.7 or 333.3 mg per gram of powder) Gentamicin has been the most commonly administered drug in this class used in veterinary medicine. The common clinical approach is to rely on gentamicin for IV, IM, or SC administration when routine use of an aminoglycoside is indicated. In some instances (for example, to broaden the spectrum) it may be administered with a β-lactam antibiotic (e.g., penicillin, ampicillin, or a cephalosporin). Examples of gentamicin dosages are listed in Table 35.2. Representative pharmacokinetic data for gentamicin in animals are shown in Table 35.4.

Intramuscular injection is a reliable route of delivery as the absorption (bioavailability, F) of gentamicin from IM sites is high, usually approaching 90% or higher in most species (Jernigan et al., 1988e; Haddad et al., 1985b, 1986; Wilson et al., 1989; Pedersoli et al., 1989, 1990; Bird et al., 1983) and bioavailability from SC sites is similar to IM bioavailability (Gilman et al., 1987; Jernigan et al., 1988e; Wilson et al., 1989). Subcutaneous administration is acceptable but the maximum concentration after SC administration is usually lower and occurs later after injection than that observed after an equivalent IM dose (Jernigan et al., 1988a; Wilson et al., 1989), which is most likely due to less blood flow to the SC injection sites than to the IM injection sites, resulting in a slower rate of absorption but not altering the extent of absorption. Systemic availability from intrauterine (IU) administration is 30% in normal cows, with maximum plasma concentrations of 3.70 μg/ml and 17.5 μg/ml being observed 30 minutes after IU doses of 2 and 4 mg/kg, respectively (al-Guedawy et al., 1983).

Effect of Age on Disposition of Gentamicin

As discussed in the disposition section earlier, neonatal animals have a larger proportion of their body weight as extracellular fluid; therefore, gentamicin volume of distribution (Vd) is larger in immature animals than in adults. The difference is usually at least twofold higher in young animals compared to adults (Riviere and Coppoc, 1981a; Sojka and Brown, 1986; Riviere et al., 1983; Cummings et al., 1990; Clarke et al., 1992).

Because systemic clearance of gentamicin relies on kidney function, this also is affected by age and is typically lower in neonates (Sojka and Brown, 1986; Sweeney et al., 1992; Martin et al., 1998; Frazier et al., 1988; Riond et al., 1986). Clearance is correlated with the plasma creatinine concentration (Sweeney et al., 1992; Martin et al., 1998).

Effect of Body Condition and Disease on Gentamicin Disposition

Because gentamicin and other drugs from this class are water soluble, dehydration reduces the apparent volume of distribution (Hunter et al., 1991; LeCompte et al., 1981). Likewise, gentamicin does not distribute to fat and obese animals will have a lower apparent volume of distribution than lean animals (Wright et al., 1991). Dose adjustments should be considered when administering gentamicin to obese animals, extremely lean animals, and animals with fluid accumulations (e.g., ascites).

In endotoxemic animals, there was decreased plasma gentamicin concentrations in dogs and cats by approximately 20–30% (Pennington et al., 1975; Jernigan et al., 1988c). However, differences between healthy and febrile goats were not significant (Ahmad et al., 1994). In a population pharmacokinetic model across species, the covariate of fever seemed to influence gentamicin volume of distribution (Martin-Jimenez and Riviere, 2001). Other

Table 35.4 Single-dose intravenous serum or plasma pharmacokinetics of gentamicin in various species. Source: Adapted from Brown and Riviere, 1991.

Species	Dose (mg/kg)	Volume of Distribution (l/kg)	Clearance (ml/min/kg)	Half-life (β) (hour)	Half-life (γ) (hour)	Reference
Dogs (juvenile)	10	0.354 (0.036)	4.08 (0.62)	1.01 (0.12)	N/A	Riviere and Coppoc, 1981a
Dogs	10	0.38 (0.029)	4.20 (0.70)	1.05 (0.13)	N/A	Riviere et al., 1981a; Riviere et al., 1981b
Dogs	10	0.30 (0.06)	3.44 (0.38)	1.01 (0.08)	N/A	Rivierie et al., 1981a; Riviere et al., 1981b
Dogs	10	0.335 (0.094)	2.94 (0.67)	1.36 (0.09)	N/A	Baggot, 1977
Dogs	4.4	0.227 (0.076)	2.27 (0.41)	1.09[a]	N/A	Brown et al., 1991
Dogs	4	0.255	3.33	1.06	N/A	Batra et al., 1983
Dogs	3	NR	2.29 (0.48)	0.91 (0.25)	N/A	Wilson et al., 1989
Cats	4.4	0.190	1.61	1.36	N/A	Short et al., 1986
Cats	5	ND	1.38 (0.35)	1.25 (0.30)	86[a]	Jernigan et al., 1988e
Cows	5	0.19 (0.04)	1.32 (0.17)	1.83 (0.18)	N/A	Haddad et al., 1986
Cattle (1 day old)	4.4	0.393 (0.040)	1.92 (0.43)	2.49 (0.73)	N/A	Clarke et al., 1985
Cattle (5 days old)	4.4	0.413 (0.050)	2.44 (0.34)	1.99 (0.33)	N/A	Clarke et al., 1985
Cattle (10 days old)	4.4	0.341 (0.021)	2.02 (0.27)	1.97 (0.21)	N/A	Clarke et al., 1985
Cattle (15 days old)	4.4	0.334 (0.039)	2.10 (0.32)	1.85 (0.13)	N/A	Clarke et al., 1985
Cattle (4–5 weeks old)	3	1.95 (1.24)	4.9 (1.9)	3.96 (1.67)	N/A	Ziv et al., 1982
Cattle (adult)	4.4	0.140 (0.020)	1.29 (0.26)	1.26 (0.19)	N/A	Clarke et al., 1985
Horse (mare)	6.6	0.21	1.1	2.2	ND	Santschi and Papich, 2000.
Horse (clinical)	4.4	0.17	1.2	1.61	ND	Tudor et al., 1999
Horse (clinical)	6.6	0.17	1.3	1.47	ND	Tudor et al., 1999
Horse	2.2	0.46	ND	0.83	ND	Godber et al., 1995
Horse	6.6	0.115	ND	0.78	ND	Godber et al., 1995
Horse (foal)	4.0	0.32–0.38	1.7–3.7	1–2.1	ND	Cummings et al., 1990
Horse (adult)	4.0	0.17	1.7	1.1	ND	Cummings et al., 1990
Horse	2.2	0.18	1.1	1.82–1.96	ND	Jones et al., 1998
Horse	2.2	0.48	1.2	4.4	ND	Whittem et al., 1996
Horse	6.6	0.19	0.95	2.3	ND	Magdesian et al., 1998
Horses	5	0.254 (0.031)	2.54 (0.33)	2.54 (0.33)	N/A	Pedersoli et al., 1980
Horses (2–3 months old)	4.5	ND	1.65 (0.79)	3.23 (0.62)	N/A	Riviere et al., 1983
Horses	2.2	ND	0.87 (0.05)	3.85 (0.40)	N/A	Bowman et al., 1986
Horses	2.2	ND	0.68 (0.17)	3.51 (0.59)	142 (31)	Bowman et al., 1986
Horses	3	0.202 (0.028)	1.41 (0.19)	1.66 (0.06)	N/A	Wilson et al., 1983
Ponies	5	0.20 (0.01)	1.27 (0.18)	1.82 (0.22)	N/A	Haddad et al., 1985b
Mammoth asses	2.2	0.12 (0.025)	1.22 (0.18)	2.07	ND	Miller et al., 1994
Sheep	2.2	0.194 (0.059)	1.56 (0.40)	1.44 (0.085)	N/A	Wilson et al., 1981
Sheep	3	ND	0.660 (0.256)	1.33[a]	41.9 (18.5)	Brown et al., 1986b
Sheep	10	ND	1.03 (0.015)	2.4 (0.5)	30.4 (18.9)	Brown et al., 1985b
Sheep	10	ND	0.805 (0.317)	1.72[a]	88.9 (19.8)	Brown et al., 1986b
Sheep	20	ND	0.882 (0.342)	1.77[a]	167.2 (42.7)	Brown et al., 1986b
Sheep (Desert)	3	0.27	0.07	4.20	ND	Elsheikh et al., 1997
Goat	3	0.22	0.08	1.041	ND	Elsheikh et al., 1997
Pigs	2	0.32 (0.032)	1.66 (0.12)	1.9 (1.47–4.89)	20.2 (13.9–34.6)	Riond and Riviere, 1988
Pigs (newborn)	5	ND	ND	5.19	ND	Giroux et al., 1995
Pigs (42 days)	5	ND	ND	3.50	ND	Giroux et al., 1995
Rabbits	20	ND	2.90–4.0	0.98–1.15	11.4–15.1	Huang et al., 1979
Rabbits	3.5	ND	2.82 (0.97)	0.74	ND	Ogden et al., 1995

(*continued*)

Table 35.4 (*Continued*)

Species	Dose (mg/kg)	Volume of Distribution (l/kg)	Clearance (ml/min/kg)	Half-life (β) (hour)	Half-life (γ) (hour)	Reference
Hawks	10	0.24 (0.03)	2.09 (0.16)	1.35 (0.18)	N/A	Bird et al., 1983
Owls	10	0.23 (0.02)	1.41 (0.10)	1.93 (0.24)	N/A	Bird et al., 1983
Eagles	10	0.21 (0.01)	1.01 (0.06)	2.46 (0.32)	N/A	Bird et al., 1983
Catfish	1	0.156	0.126	12.2	N/A	Setzer, 1985
Catfish	10	0.176	0.215	11.87	N/A	Rolf et al., 1986
Guinea pigs	40	ND	3.4	1.01	1.01	Chung et al., 1982
Buffalo calves	5	0.43	54.61	5.69	ND	Garg et al., 1991a, 1991b
Turkeys	5	0.190	49.8	2.570	ND	Pedersoli et al., 1989
Roosters	5	0.228 (0.019)	0.775 (0.132)	3.38 (0.62)	N/A	Pedersoli et al., 1990
Turtles	3	ND	ND	40–44	ND	Beck et al., 1995

Values reported as arithmetic mean followed by SD or SEM in parentheses. N/A, not applicable (inappropriate term for the model used); ND, not determined; NR, not reported.
[a] Harmonic mean; data are IV and IM data pooled together.

conditions that have been shown to alter gentamicin disposition include endocrinopathies, pregnancy, and other concurrent drug administrations.

Amikacin

Amikacin is approved for animals as a 50 mg/ml injectable solution (Amikin, Amiglyde) as well as an intrauterine infusion (250 mg/ml intrauterine solution) for horses. Tables 35.5, 35.6, and 35.7 list some selected pharmacokinetic parameters for amikacin in various species of animals. It is common to initially rely on gentamicin for routine aminoglycoside treatment, but when resistance is observed or suspected, amikacin should be considered because resistance is uncommon for amikacin. Amikacin is particularly important for treating infections caused by *E. coli* and *Klebsiella pneumoniae* that have acquired multidrug resistance from extended spectrum β-lactamase (ESBL). Amikacin is often one of the few agents, other than a carbapenem, that is active against these bacteria.

Amikacin shows in vitro activity against *Staphylococcus* spp., although it is not a common agent for treatment of staphylococcal infections. Many guidelines recommend addition of a β-lactam antibiotic for treating infections caused by *Staphylococcus* spp. Amikacin is considered for treating methicillin-reisistant *Staphylococcus pseudintermedius* infections of the skin and soft tissue. However, there may be an association between amikacin resistance and methicillin resistance in these isolates (Gold et al., 2014).

Like gentamicin, amikacin is hydrophilic and is rapidly taken up by either IM or SC administration with bioavailability of approximately 90% or higher (Gronwall et al.,

Table 35.5 Pharmacokinetic data for amikacin in horses, foals, and dogs

		Half-life (hour)	Volume of Distribution (l/kg)	Clearance (ml/kg/min)	Mean residence time (hour)
Compilation of 10 data sets from 44 adult horses; average dose 8.3 mg/kg	Mean Std. Dev	1.83 0.75	0.214 0.076	1.45 0.45	2.50 0.565
Compilation of 6 data sets from 37 foals, 1–11 days of age; average dose 20 mg/kg	Mean Std. Dev	4.43 1.09	0.68 0.19	1.83 0.40	5.4 0.22
Compilation of 9 data sets from 42 dogs; average dose 13.6 mg/kg	Mean Std. Dev	1.0 0.24	0.22 0.11	2.40 0.77	1.50 0.92

Data from: Pinto et al., Equine Vet J. 43, 112–116, 2011; Orsini JA, et al., Can Vet J 37, 157–160, 1996; Brown MP et al., Am J Vet Res. 45: 1610–1613, 1984; Horspool et al., J Vet Pharmacol Therap. 17: 291–298, 1994; Orsini et al., J Vet Pharmacol Therap. 8: 194–201, 1985; Magdesian et al., Am J Vet Res. 65: 473–479; 2004; Golenz MR et al., Equine Vet J. 26: 367–373, 1994; Bucki et al., J Vet Intern Med. 18: 728–733, 2004; KuKanch and Coetzee, J Vet Pharmacol Therap. 31:102–107, 2008; Cabana and Taggart, Antim Agent Chemo. 3: 478–483, 1973; Baggot et al., Am J Vet Res. 46: 1793–1796, 1985.

Table 35.6 Single-dose intravenous pharmacokinetics of amikacin in various species (horses and dogs see Table 35.5). Source: Adapted from Brown and Riviere 1991.

Species	Dose (mg/kg)	Volume of distribution (l/kg)	Clearance (ml/min/kg)	Half-life (hour)	Reference
Cats	5	0.134 (0.008)	110 (15)	NR	Shille et al., 1985
Cats	10	0.14 (0.008)	121 (22)	NR	Shille et al., 1985
Cats	20	0.18 (0.022)	138 (2.6)	NR	Shille et al., 1985
Cats	5	NR	1.46 (0.26)	79[a] (19)	Jernigan et al., 1988c
Calves	7.5	350	1.5	150.5	Carli et al., 1990
Sheep	7.5	200	0.7	115.5	Carli et al., 1990
African grey parrots	5	289	188	1.06	Gronwall et al., 1989
African grey parrots	10	184	142	0.90	Gronwall et al., 1989
African grey parrots	20	444	229	1.34	Gronwall et al., 1989

NR, not reported.

[a] Harmonic mean (±SD).

1989; Bloomfield et al., 1997; Jernigan et al., 1988d; Cabana and Taggart, 1973; Ziv, 1977; Baggot et al., 1985; Carli et al., 1990).

Amikacin is also used in reptiles (snakes, turtles) and has a rapid absorption, but slow renal clearance. In these animals, clearance of amikacin (like many other drugs in cold-blooded animals) is temperature dependent.

Pharmacokinetics have been studied in adult horses and foals. Pharmacokinetic data from various studies are summarized in Table 35.5. As for gentamicin, the volume of distribution in neonatal foals is much higher than adults, necessitating higher clinical doses (by at least two times) compared to adults.

Kanamycin

Kanamycin is among the least active aminoglycosides in comparison to gentamicin and amikacin. Subsequently, the clinical use of kanamycin has fallen out of popularity in veterinary medicine in recent years. For example, against clinical isolates of *Pseudomonas aeruginosa* the highest rate of resistance was for kanamycin (90% resistant) compared to gentamicin (7%) and amikacin (3%) (Rubin et al., 2008).

Because kanamycin has chemical properties that are similar to other aminoglycosides (amikacin is synthesized from kanamycin), pharmacokinetic properties are

Table 35.7 Nonintravenous disposition values for amikacin in various species (means with standard deviations in parentheses). Source: Adapted from Brown and Riviere 1991.

Species	Dose (mg/kg)	Route	Half-life (hour)	F (%)	Reference
Horses	4.4	IM	NR	100	Orsini et al., 1985
Horses	6.6	IM	NR	100	Orsini et al., 1985
Horses	11	IM	NR	100	Orsini et al., 1985
Cats	5	IM	NR	NR	Shille et al., 1985
Cats	10	IM	NR	NR	Shille et al., 1985
Cats	20	IM	NR	NR	Shille et al., 1985
Cats	5	SC	NR	NR	Shille et al., 1985
Cats	10	SC	NR	NR	Shille et al., 1985
Cats	20	SC	NR	NR	Shille et al., 1985
Cats	5	IM	119	90 (36)	Jernigan et al., 1988d
Cats	5	SC	118	100 (19)	Jernigan et al., 1988d
Sheep	7.5	IM	1.96	87	Carli et al., 1990
Calves	7.5	IM	1.94	99	Carli et al., 1990
African grey parrot	5	IM	1.08	98	Gronwall et al., 1989
African grey parrot	10	IM	1.04	61	Gronwall et al., 1989
African grey parrot	15	IM	0.97	106	Gronwall et al., 1989
Gopher snake (25° C)	5	IM	1.2 (0.17)	ND	Mader et al., 1985
Gopher snake (37° C)	5	IM	1.25 0.5)	ND	Mader et al., 1985

ND, not determined; NR, not reported.

also similar. Absorption from IM or SC injection is complete and rapid, volume of distribution resembles the volume of extracellular fluid, and excretion from the kidneys is close to the glomerular filtration rate for that species.

Apramycin

Apramycin, an aminoglycoside derived from *Streptomyces tenebrarius* (Ryden and Moore, 1977), is available for veterinary use, but it has limited use. The only formulations currently approved in the US of this drug are powder (Apralan) to be added to feed (Type A medicated feed article) at 150 grams per ton of feed, and the soluble powder to be added to water (100 mg per liter) to deliver 12.5 mg/kg for 12 days of treatment in medicated water. For each formulation, the indication is to treat porcine colibacillosis (pig scours) caused by *Escherichi coli*. More details on apramycin in selected species are presented in Table 35.8.

Tobramycin

Tobramycin is produced by *Streptomyces tenebrarius* and is structurally similar to kanamycin. Tobramycin is not extensively used in veterinary medicine, although it is used occasionally in dogs and cats because of its good activity against most *Pseudomonas aeruginosa* organisms. Typically, amikacin is used more often when it is necessary to treat resistant infections. However, occasionally amikacin has been unavailable because of manufacturing shortages and tobramycin has been used as a substitute.

Pharmacokinetic properties (absorption characteristics, volume of distribution, clearance, and half-life) are similar to other aminoglycosides. In cats, tobramycin systemic clearance was 2.21 ± 0.59 and 1.69 ± 0.36 ml/min/kg after doses of 5 mg/kg and 3 mg/kg IV, respectively, and a Vd(ss) of 0.19 ± 0.03 and 0.18 ± 0.03 l/kg, respectively (Jernigan et al., 1988b). Bioavailability after IM and SC tobramycin administration in cats was rapid and complete (Jernigan et al., 1988d). Urine tobramycin concentrations following 2.2 mg/kg three times a day were 66 ± 39 μg/ml when urine was obtained as a 6-hour collection in dogs (Ling et al., 1981). In horses (Newman et al., 2013), after a dose of 4 mg/kg it had a volume of distribution of 0.18 l/kg, and elimination half-life of 4.6 hours, with a clearance of 1.2 ml/kg/min. It was over 80% absorbed from IM injection.

After IV administration to camels (Hadi et al., 1994), tobramycin (1.3 mg/kg) elimination half-life was 189 minutes. The apparent Vd (area method) was 245 ml/kg and Vd(ss) was 228 ml/kg. Clearance was measured at 0.9 ml/min/kg. After a 1.0 mg/kg IM dose of tobramycin, bioavailability was almost 91%, with an elimination half-life of 201 minutes.

Neomycin

Most neomycin is used topically or administered orally (e.g., Biosol used for enteritis caused by *Escherichia coli*) to achieve a local effect in the intestine. Approved formulations are powder for addition to feed at 715 grams per kg of feed (Neomix), or neomycin oral solution (200 mg/ml) to be added to drinking water, both the oral solution and feed additive are designed to deliver 22 mg/kg up to 14 days. The oral solution also may be administered directly to individual animals.

Pharmacokinetic information about neomycin's use in human and veterinary medicine is limited because systemic use is practically nonexistent, but some details are available in previous editions of this book.

Dihydrostreptomycin and Streptomycin

The clinical use of dihydrostreptomycin and streptomycin has declined substantially in veterinary medicine. An old formulation of penicillin–dihydrostreptomycin (Pen-Strep) is off the market in North America. There is still a formulation containing dihydrostreptomycin sulfate (500 mg/ml) registered for treatment of *Leptospira*

Table 35.8 Selected pharmacokinetic parameters of apramycin

Species	Volume of distribution (l/kg)	Clearance (l/kg/h)	Half-life (hour)	Reference
Sheep	0.167	0.078	90.96	Lashev et al., 1992
Cow (lactating)	1.263	12.164[a]	2.10	Ziv et al., 1995
Ewe (lactating)	1.446	14.142[a]	1.85	Ziv et al., 1995
Goat (lactating)	1.357	11.68[a]	2.14	Ziv et al., 1995
Rabbits	0.284	0.258	48.06	Lashev et al., 1992
Adult chickens	0.182	0.078	100.54	Lashev et al., 1992
18-day-old chicks	0.254	0.218	48.0	Lashev et al., 1992
Japanese quail	0.133[b]	0.186	0.50	Lashev and Mihailov, 1994
Pigeons	0.077	0.210	15.24	Lashev et al., 1992

[a] Value in ml/kg/min.

[b] V_α is area, not steady state.

in dogs, horses, pigs, and ruminants. Although dihydrostreptomycin has been used in the treatment of cows infected with *Leptospira interrogans* serovar *hardjo* subtype *hardjobovis* (Gerritsen et al., 1994), this use is not common and the product is not marketed.

There is an oral formulation of streptomycin (250 mg/ml) still registered for oral treatment of bacterial enteritis in pigs, cattle, and poultry. It has been administered directly or added to drinking water. Because the use of these products is uncommon, previous editions of this book should be consulted for more detailed information on their use.

After IM doses of 5.5 mg/kg dihydrostreptomycin, maximum concentrations ranged from 5.1 to 17.0 μg/ml, with peak concentrations occurring earlier and more variable from the commercial preparation containing procaine penicillin G, dihydrostreptomycin, dexamethasone, and chlorpheniramine than from the commercial product containing only dihydrostreptomycin and procaine penicillin G (Rollins et al., 1972). Half-lives range from 2.35 to 4.50 hours. Because streptomycin and dihydrostreptomycin are chemically very similar, their dispositions also may be nearly identical.

Paromomycin

Paromomycin is a wide-spectrum aminoglycoside antibiotic produced by *Streptomyces rimosus* var. *paromomycinus* and, unlike others in this class, has both gram-positive and gram-negative activity. Paromomycin is poorly absorbed from the gastrointestinal tract, which is clearly an advantage if used to treat certain bacterial or protozoal gastrointestinal infections. The pharmacokinetics of paromomycin in the dog has been described by Belloli et al. (1996); see Table 35.9.

Table 35.9 Pharmacokinetic parameters of paromomycin in the dog. Source: Belloli et al., 1996.

Parameter	IV	IM	SC
Half-life (hour)	91.03	114.22	120.86
VD (l/kg)	0.51	ND	ND
VD_{ss} (l/kg)	0.33	ND	ND
Clearance (l/min/kg)	0.0037	ND	ND
C_{max} (μg/ml)	ND	32.1	36.3
F	ND	>0.99	>0.99
MRT (min)	98.7	204.8	203.8
K_{el} (min^{-1})	0.0186	0.0061	0.0057

ND, not determined.

Giardia, Leishmania, Entamoeba histolytica, and *Balantidium coli* are susceptible to paromomycin (Barr et al., 1994; Belloli et al., 1996). Paromomycin has been used to treat cryptosporidiosis in a cat (Barr et al., 1994) and leishmaniasis (*Leishmania infantum*) in dogs (Poli et al., 1997). However, a retrospective case study in cats treated with high-dose oral paromomycin (165 mg/kg) suggested that 4 of 31 individuals developed acute nephrotoxicity, deafness, and/or possible cataract formation (Gookin et al., 1999), implying that enough oral absorption occurred for this large and highly charged aminoglycoside to exert an adverse effect. Therefore, use of this drug at these high doses should be approached with caution until further data are available.

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36

Chloramphenicol and Derivatives, Macrolides, Lincosamides, and Miscellaneous Antimicrobials

Mark G. Papich

Drugs listed in this chapter do not warrant a separate chapter and are included together, as they comprise narrow uses in veterinary medicine. They have some features in common – for example they inhibit protein synthesis in bacteria (with macrolides, lincosamides, and chloramphenicol acting at a similar site), and have some similar pharmacokinetic features.

Some of these drugs are not as common or available as in previous years. Some older drugs have given way to newer derivatives and their discussion has been greatly abbreviated in this edition of the book. Older editions may be consulted for more detailed and historical information.

Chloramphenicol

Chemical Features

Chloramphenicol chemically is D-(-)-threo-1-*p*-nitrolphenyl-2-dichloroacetamido 1,3-propanediol (Figure 36.1), has a pK_a of 5.5, and was first isolated from the soil organism *Streptomyces venezuelae* in 1947. The chloramphenicol used today is manufactured synthetically. Chloramphenicol is slightly soluble in water and freely soluble in propylene glycol and organic solvents. Chloramphenicol is a broad-spectrum antibiotic, inhibiting gram-positive and gram-negative organisms, aerobic and anaerobic bacteria, and many intracellular organisms. Chloramphenicol has three functional groups that largely determine its biological activity: the *p*-nitrophenol group, the dichloroacetyl group, and the alcoholic group at the third carbon of the propanediol chain (Yunis, 1988). Replacement of the *p*-NO_2 group by a methylsulfonyl (HC_3-SO_2) moiety produces thiamphenicol and a substantial change in biological activity, while modification of the propanediol group by the addition of a fluorine atom produces florfenicol. Both of these are discussed in more detail in Sections Thiamphenicol and Florfenicol. Loss of the dichloroacetyl group altogether results in loss of biological activity (Yunis, 1988; Hird and Knifton, 1986).

After the discovery of chloramphenicol in 1947 it was in popular use decades ago, but has been gradually replaced by safer alternatives. The small animal formulation is approved by the FDA (Chloromycetin) but is not actively marketed. The use of chloramphenicol diminished in the 1970s and 80s because other active and safer drugs became available. Today, chloramphenicol has experienced a bit of a resurgence in companion animal medicine. Multidrug resistant bacteria, particularly methicillin-resistant *Staphylococcus* spp., are usually susceptible to chloramphenicol and this is one of the most common drugs selected for use by small animal veterinarians (Papich, 2012; Bryan et al., 2012; Frank and Loeffler, 2012). Antibiotic-resistant *Enterococcus* spp. are also often susceptible. Chloramphenicol has the disadvantage of a narrow margin of safety in dogs and cats, and necessity of frequent administration in dogs to maintain adequate concentrations (three or four times daily oral administration). These disadvantages still exist, but the activity of chloramphenicol against bacteria (e.g., staphylococci) that are resistant to other oral drugs has created increased use of chloramphenicol in recent years.

Drug Formulations

Many formulations have been removed from the commercial market because chloramphenicol no longer is in wide use for humans. Chloramphenicol has FDA approval for use in dogs, and is available in 100, 250, and 500 mg tablets (Chloromycetin). The oral suspension of chloramphenicol palmitate is rarely available. Chloramphenicol is not soluble and injectable formulations include esters such as succinate and palmitate, glycinate, or undecylenate. There also has been a propylene glycol solution. None of the injectable formulations are used today. Although chloramphenicol is poorly soluble (<5 mg/ml), the poor solubility does not interfere with oral absorption. Chloramphenicol is absorbed orally

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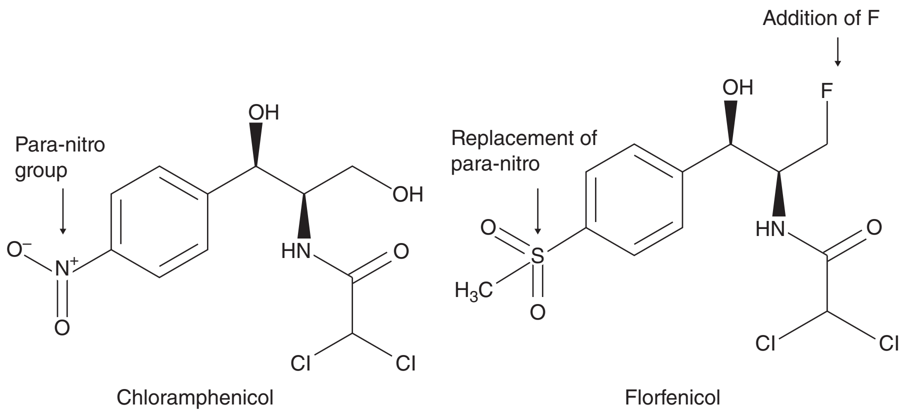


Figure 36.1 The chemical structure of chloramphenicol and modifications to form florfenicol.

with or without food (except some formulations in cats). Tablets and capsules have similar oral absorption in dogs. Topical formulations of chloramphenicol have been used for otic and ophthalmic use, but the otic formulations have been replaced by newer forms containing florfenicol.

Mechanism of Action

Chloramphenicol inhibits protein synthesis. Its biological activity is due to interference with peptidyltransferase activity at the 50S ribosomal subunit, which is near the site of action of macrolide antibiotics and for which there can be competition (Yunis, 1988). Because of the interaction with peptidyltransferase, binding with the amino acid substrate cannot occur, and peptide bond formation is inhibited. Chloramphenicol affects mammalian protein synthesis to some degree, especially mitochondrial protein synthesis. Mammalian mitochondrial ribosomes have a strong resemblance to bacterial ribosomes (both are 70S), with the mitochondria of the bone marrow especially susceptible. Prolonged administration to animals has been associated with a dose-related bone marrow suppression, especially in cats (Watson, 1980).

The action of chloramphenicol (and florfenicol) is regarded as bacteriostatic, rather than bactericidal (Maaland et al., 2015). There are isolated examples in which bactericidal effects have been observed, but chloramphenicol and similar drugs in this class usually behave as bacteriostatic agents and the drug concentration in animals should be maintained above the MIC for as long as possible during the dose interval.

Spectrum of Activity

Chloramphenicol has a broad spectrum of activity. It is active against *Staphylococcus pseudintermedius*, *S. aureus,* streptococci, and some gram-negative bacteria, such as *Pasteurella multocida*, *Mannheimia haemolytica*, and *Histophilus somni. Escherichia coli, Proteus* spp., and *Salmonella* spp. may be susceptible, but resistance can occur with many gram-negative bacteria, especially the Enterobacteraceae. One reason for the increased use of chloramphenicol, especially in dogs, is that it has retained activity against *Staphylococcus pseudintermedius,* including methicillin-resistant strains (Perreten et al., 2010). However, resistance by staphylococci may occur from repeated administration. Anaerobic bacteria, *Mycoplasma* spp., and many Rickettsiae also are susceptible. The Clinical Laboratory Standards Institute (CLSI, 2015) approved break point for susceptibility is ≤4 μg/ml for streptococci and ≤8 μg/ml for other organisms (Watts et al., 1999).

Bacterial Resistance

Four mechanisms of resistance to chloramphenicol have been described (Yunis, 1988; Schwarz et al., 2004). The most important is plasmid mediated due to the presence of the chloramphenicol acetyltransferase enzyme, which catalyzes a reaction that causes enzymatic inactivation by acetylation of the drug. This can occur through different types of chloramphenicol acetyltransferases (Schwarz et al., 2004). The acetyltransferases that cause resistance to chloramphenicol are less likely to affect florfenicol, making florfenicol more active against some pathogens (discussed in Section Florfenicol). Other mechanisms of resistance include efflux systems, inactivation by phosphotransferases, decreased bacterial cell wall permeability, altered binding capabilities at the 50S ribosomal subunit, and inactivation by nitroreductases.

Pharmacokinetics

Absorption and Distribution

The pharmacokinetic parameters of chloramphenicol have been studied in several animal species and are summarized in Tables 36.1 and 36.2. Chloramphenicol in animals is well absorbed via both oral and parenteral routes, with a few notable species exceptions. Plasma half-lives vary, ranging from 0.9 hours in ponies to 5.1 hours in the

Table 36.1 Chloramphenicol pharmacokinetics in dogs (compilation of nine studies)

		Data set										
		1	2	3	4	5	6	7	8	9	Mean	SD
Breed		Mixed	Beagle	Ghound	large dog	small dog	Ghound	Ghound	Ghound	Ghound		
n		4	6	6	6	6	4	5	4	6		
Formulation (50 mg/kg)		capsule	capsule	capsule	capsule	capsule	capsule	capsule	tablet	capsule		
Elimination rate	1/hour	0.42	0.52	0.40	0.18	0.54	0.14	0.23	0.22	0.40	0.34	0.15
Half-life	hour	1.64	1.35	1.75	3.85	1.29	4.82	2.99	3.19	1.75	2.51	1.25
T_{max}	hour	4.00	2.00	3.00	3.00	1.50	2.00	3.00	1.50	3.00	2.56	0.85
C_{max}	µg/ml	16.70	19.65	18.60	27.50	20.00	16.50	18.50	23.80	18.60	19.98	3.54
AUC	h*µg/ml	97.79	69.85	109.91	191.15	82.44	89.52	114.95	110.62	109.91	108.46	34.51
V/F	ml/kg	1212.94	1389.17	1149.22	1454.03	1124.29	3884.62	1874.17	2081.52	1149.22	1702.13	886.07
Cl/F	ml/h/kg	511.33	715.87	454.90	261.57	606.47	558.53	434.95	451.99	454.90	494.50	126.82
MRT	h	4.63	3.00	4.28	5.94	3.42	5.73	5.75	5.43	4.28	4.72	1.07

Ghound, Greyhound; T_{max}, time to peak concentration; C_{max}, peak concentration; AUC, area under the curve; V/F, volume of distribution per fraction absorbed orally; CL/F, clearance per fraction absorbed; MRT, mean residence time, SD, standard deviation.

Data set sources: (1) Eads, 1952; (2) Mercer, 1971; (3, 4, 5) Watson, 1974; (6) Watson, 1972a; (7) Watson, 1972b; (8) Watson and McDonald, 1976; (9) Watson, 1973.

cat (Davis et al., 1972). Fasted cats showed differences in absorption between the chloramphenicol tablets and the chloramphenicol palmitate suspension (Watson 1992). The liquid formulation showed a lower systemic drug availability, indicating that hydrolysis of the palmitate form is necessary and that there is a higher risk of drug failure when the palmitate suspension is used to treat sick cats that are also not eating. In ruminants, microflora present in the ruminant forestomach tend to metabolize chloramphenicol faster than it can be absorbed, making chloramphenicol administered orally of little use in ruminant animals. This point is rather moot since administration of chloramphenicol to food animals in the United States is currently illegal (discussed in more detail in Chapter 55). In most animals, 30–46% of chloramphenicol is bound to plasma proteins, leaving much of the drug in the free and active form. Chloramphenicol is widely distributed to many tissues of the body due to its nonionized state and high lipophilicity, enabling it to cross lipid bilayers quite easily. The volume of distribution (Vd) is typically greater than 1.0 l/kg and has been measured at 1–2.5 l/kg (Tables 36.1 and 36.2). Chloramphenicol reaches sufficient concentrations in most tissues of the body, including the eye, central nervous system (CNS), heart, lung, prostate, saliva, liver, and spleen, among others (Ambrose, 1984; Hird and Knifton, 1986). Chloramphenicol concentrations in cerebrospinal fluid (CSF) are approximately 50% of corresponding plasma concentrations. Chloramphenicol can also cross the placental barrier in pregnant animals and can diffuse into the milk of nursing animals.

Metabolism and Excretion

Chloramphenicol is metabolized by the liver after absorption into the systemic circulation. One of the largest drawbacks to chloramphenicol administration is the rapid metabolic clearance, producing short half-lives in many species and necessity for frequent administration. As shown in Table 36.1 for dogs, the short half-life translates to a need to be administered three times daily. In horses, because of rapid elimination rates, tissue fluid concentrations persisted for only 3 hours after IV administration of chloramphenicol sodium succinate (Brown et al., 1984). Phase II glucuronidation is the principal pathway for the hepatic biotransformation of chloramphenicol, with the principal metabolite being chloramphenicol glucuronide. A few hydrolysis products have also been identified. Cats excrete chloramphenicol more slowly than other animals, perhaps owing to the cat's deficiency in some glucuronidase enzymes. One report notes that 25% of the total dose of chloramphenicol is excreted in the urine in the active form in cats compared to 6% in dogs (Hird and Knifton, 1986). Most of the absorbed chloramphenicol (approximately 80%) is excreted into the urine as inactive metabolites via tubular secretion.

The effect of age on clearance of chloramphenicol is inconsistent. Calves showed differences compared to older cattle, but this is probably irrelevant because it should not be used in food animals (Burrows et al., 1984). Brumbaugh et al. (1983) found that in neonatal horses, elimination and Vd did not differ from adults. Bioavailability in foals was 83%, with an oral half-life of 2.54 hours.

Adverse Effects and Precautions

Bone marrow suppression has been the most important adverse effect associated with chloramphenicol administration to people. Bone marrow injury from chloramphenicol takes two forms (Yunis, 1988). The first type

Table 36.2 Selected serum pharmacokinetic parameters of chloramphenicol in animals

Species	Dose (mg/kg)	Route	Formulation	Half-life (hr)	Volume of distribution (l/kg)	Comments	Reference
Cats	22	IV	Base	5.1	2.36	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
Sheep	30	IV	Base	1.702	0.691		Dagorn et al., 1990
	30	SC	Base	17.93	NA		Dagorn et al., 1990
	30	IM	Base	2.71	NA		Dagorn et al., 1990
Adult swine	22	IV	Base	1.3	1.05	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
Piglets	25	IV	Base	12.7	0.9411	Normal piglets	Martin and Wiese, 1988
	25	IV	Base	17.2	0.9549	Colostrum-deprived piglets	Martin and Wiese, 1988
Goats	25	IV	Succinate	1.22	1.683	Nonfebrile animals	Kume and Garg, 1986
	25	IV	Succinate	1.29	1.962	Febrile animals	Kume and Garg, 1986
	25	IM	Succinate	1.46	3.019	Nonfebrile animals	Kume and Garg, 1986
	25	IM	Succinate	1.45	2.769	Febrile animals	Kume and Garg, 1986
	22	IV	Base	2.0	1.33	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
	10	IV	Succinate	1.47	0.312	Normal animals	Abdullah and Baggot, 1986
Goats	10	IV	Succinate	3.97	0.287	Starved animals	Abdullah and Baggot, 1986
	22	IV	Base	2.0	1.33	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
	10	IV	Succinate	1.47	0.312	Normal animals	Abdullah and Baggot, 1986
	10	IV	Succinate	3.97	0.287	Starved animals	Abdullah and Baggot, 1986
Cattle	40	IV	Base	2.81	0.351		Sanders et al., 1988
	90	IM	Base	1.345	NA	2 doses 48 hours apart	Sanders et al., 1988
	90	SC	Base	1.153	NA	2 doses 48 hours apart	Sanders et al., 1988
Calves	30	IV	Base	3.98	1.208	Age not reported; average weight 73 kg	Guillot and Sanders, 1991
1-day-old calves	25	IV	Base in PG vehicle	7.56	1.031		Burrows et al., 1983
7-day-old calves	25	IV	Base in PG vehicle	5.96	0.808		Burrows et al., 1983
14-day-old calves	25	IV	Base in PG vehicle	4.0	0.903		Burrows et al., 1983
28-day-old calves	25	IV	Base in PG vehicle	3.69	0.69		Burrows et al., 1983
9-month-old calves	25	IV	Base in PG vehicle	2.47	1.38		Burrows et al., 1983
Horses	22	IV	Base in PG vehicle	0.51–0.78	0.86–1.26		Varma et al., 1987
Ponies	22	IV	Base	0.9	1.02	Dissolved in 50% aqueous solution of *N*,*N*,di-methylacetamide	Davis et al., 1972
Foals							
1 day old	25	IV	Succinate	5.29	1.1		Adamson et al., 1991
3 days old	25	IV	Succinate	1.35	0.759		Adamson et al., 1991
7 days old	25	IV	Succinate	0.61	0.491		Adamson et al., 1991
14 days old	25	IV	Succinate	0.51	0.426		Adamson et al., 1991
42 days old	25	IV	Succinate	0.34	0.362		Adamson et al., 1991
1–9 days old	50	IV	Succinate	0.95	1.6	After oral suspension administered oral, availability was 83% and half-life of 2.54 hours	Brumbaugh et al., 1983
Rabbits	100	IV	Succinate	1.1575	NA		Mayers et al., 1991
Chickens	20	IV	Succinate	8.32	0.24	Normal animals	Atef et al., 1991a
	20	IV	Succinate	26.21	0.3	*E. coli*-infected animals	Atef et al., 1991a
	20	IM	Succinate	7.84	0.44		Atef et al., 1991a
	20	PO	Succinate	8.26	0.41		Atef et al., 1991a

NA, data not available; PG, propylene glycol.

is the most common and involves a dose-related suppression of the bone marrow precursor erythroid series. This toxicosis is reversible. The evidence suggests that this bone marrow suppression is the result of suppression of mitochondrial protein synthesis in bone marrow cells. In bone marrow cells there is vacuolation of the myeloid and erythroid series precursor cells, and inhibition of erythroid and granulocytic colony forming units (IARC, 1976, 1990). In both the dog and the cat, dose-related bone marrow suppression is possible. However, signs of toxicity reverse when chloramphenicol therapy is discontinued.

The second type of bone marrow toxicity, aplastic anemia, has been described in people but not in animals. In people, it is rare and independent of dose and treatment duration. This toxicity results in bone marrow aplasia, chiefly characterized by a profound and persistent pancytopenia. Aplastic anemia occurs in approximately 1 : 10,000 to 1 : 45,000 humans who receive chloramphenicol. It appears that the para-nitro group of the chloramphenicol molecule is responsible for this more serious form of bone marrow toxicity (Figure 36.1). The para-nitro group undergoes nitroreduction, leading to the production of nitrosochloramphenicol and other toxic intermediates, which trigger the stem cell damage in humans (IARC, 1976, 1990; Yunis, 1988). Modification of the molecule to eliminate the para-nitro group to produce either thiamfenicol or florfenicol reduces the risk of chloramphenicol-associated aplastic anemia (Figure 36.1).

Chloramphenicol-induced aplastic anemia in humans is important from a food-animal residue standpoint. If chloramphenicol is used to treat infections in food animals, it is possible that low concentrations of chloramphenicol in milk, meat, and other edible tissues from the animals will be consumed by people and cause aplastic anemia in susceptible individuals. Chloramphenicol residues have been known to persist for prolonged periods in food animals (Korsrud et al., 1987). Even though the amount consumed may be small, reaction that may occur in people are not dependent on dose. Thus, there is a public health risk for individuals consuming these products. For this and other reasons, the use of chloramphenicol in food-producing animals has been banned in the United States. The hazards of using chloramphenicol in food animals have also been reviewed by others (Settepani, 1984; Lacey, 1984).

Other adverse effects caused by chloramphenicol in animals have been observed since the drug is used more in recent years to treat drug-resistant bacteria. Young animals and cats are sensitive to intoxication due to impaired glucuronidation pathways. Cats given 60 and 120 mg/kg/day PO every 8 hours for 21 and 14 days (respectively) showed clinical signs of depression, dehydration, reduced fluid intake, weight loss, emesis, and diarrhea. Bone marrow hypoplasia was also documented in addition to pancytopenia (Watson, 1980). Other investigators (Penny et al., 1967, 1970) administered to cats 50 mg/kg/day IM, with the cats showing marked depression and inappetence by day 7 of administration, severe bone marrow changes by day 14, and becoming extremely ill by day 21.

Gastrointestinal (GI) disturbances are among the most common in dogs (Short et al., 2014; Bryan et al., 2012). Dogs may exhibit events such as vomiting, diarrhea, anorexia, drooling, gagging or any combination of these clinical signs. They tend to resolve when the medication is discontinued. These effects may be related to GI injury as oral administration resulted in intestinal mucosal damage and diarrhea in calves and reduced glucose absorption (Rollin et al., 1986). Another issue that has emerged now that chloramphenicol is used more often is peripheral neuropathy. In one report, this adverse effect was almost as common as the gastrointestinal problems (Short et al., 2014). Signs that may be observed are ataxia, rear limb weakness, trouble standing, and/or jumping, or trembling on the back legs. This is believed to be caused by peripheral neuropathy, but the cellular mechanism is unknown. One microscopic study in three dogs (Kuroda et al., 1974) identified degenerative changes in peripheral nerves. Larger-breed dogs may be at higher risk for the neuropathy based on anecdotal accounts. Most dogs recover when the medication is discontinued.

Drug Interactions

Chloramphenicol is an inhibitor of the cytochrome P450 (CYP) drug-metabolizing enzymes. Enzyme specificity has not been fully characterized, but there is evidence that one of the enzymes inhibited is canine CYP2B11 (Martinez et al., 2013). Among the drugs substrates that may be affected by inhibition from chloramphenicol are anticonvulsants (e.g., phenobarbital), propofol, benzodiazepines, and other anesthetics. For example, chloramphenicol significantly affected metabolism of methadone in dogs (KuKanich and KuKanich, 2015).

Clinical Use

The FDA-approved dose for dogs is 55.5 mg/kg oral, every 6 hours. This dose is likely to increase the risk of adverse effects and the most common clinical dose, based on pharmacokinetic studies and evidence of efficacy is 50 mg/kg every 8 hours oral. Chloramphenicol has been used for treatment of a wide range of susceptible microbial infections, including those caused by salmonellae, intracellular and extracellular bacteria, rickettsiae, and mycoplasmata; infections of the eyes and CNS; and infections due to anaerobic organisms (IARC, 1976, 1990). One of the reasons for its popularity has been the high lipophilicity. Chloramphenicol readily penetrates cells, making it active against intracellular bacteria. It can penetrate tissues that otherwise are difficult to treat, such as the CNS, which is further discussed below. Chloramphenicol was shown in one study to be equally effective for treatment of Rocky Mountain spotted fever in dogs as enrofloxacin and tetracyclines (Breitschwerdt et al., 1990). Chloramphenicol has been used to treat infections caused by *Staphylococcus* spp., streptococci, *Brucella* spp., *Pasteurella* spp., *E. coli, Proteus* spp., *Salmonella* spp., *Bacillus anthracis, Arcanobacterium pyogenes, Erysipelothrix rhusiopathiae,* and *Klebsiella pneumoniae.* It is consistently active against anaerobic bacteria.

Chloramphenicol has been suggested for treatment of infections of the CNS (encephalitis, meningitis) because it is able to cross the inflamed or uninflamed blood–brain barrier and attain therapeutic concentrations in the CSF and the brain. Despite the rationale for this use, some experts have suggested that since chloramphenicol is merely bacteriostatic against gram-negative pathogens, and there is a lack of phagocytes or immunoglobulins in CSF, chloramphenicol is not well suited to treat serious infections of the CNS (Rahal and Simberkoff, 1979).

Chloramphenicol attains high concentrations in the eye when given systemically or after topical application on the cornea and is useful in treating susceptible bacterial conjunctivitis, panophthalmitis, endophthalmitis, and bacterial diseases of the cornea (Conner and Gupta, 1973). Topical formulations are not as readily available owing to the risk of aplastic anemia (discussed in Section Adverse Effects and Precautions), which can be caused by topical exposure.

Chloramphenicol has been used to treat bacterial infections of the respiratory tract because it may have better penetration across the blood–bronchus barrier into respiratory secretions and respiratory lining fluid than more polar or less lipophilic antibiotics. Respiratory infections in horses, dogs, cats, and exotic animals are among the uses of oral chloramphenicol.

Chloramphenicol is among the few drugs that can be administered orally to horses with safety. It achieves moderate systemic absorption of 21–40% (Gronwall et al., 1986) and has no serious adverse effects on the equine digestive system. For treatment in horses, tablets or capsules are mixed with vehicles such as molasses or corn syrup to facilitate oral administration. Chloramphenicol has been administered to horses for respiratory infections, pleuritis, CNS infections, and joint infections. Because there are other active drugs available, it is most often considered as an option when bacteria are resistant to other antibiotics. The recommended doses are based on specific pharmacokinetic studies in adults and foals (Gronwall et al., 1986; Brumbaugh et al., 1983).

Chloramphenicol has been administered to exotic animals, especially reptiles and amphibians, to treat a variety of infections (Clark et al., 1985); although florfenicol (see Section Florfenicol) has taken over some of these uses. Chloramphenicol administration in 15 species of birds was examined, and the investigators concluded that after IM injections of 50 mg/kg, chloramphenicol would produce adequate concentrations to treat susceptible bacteria for 8–12 hours, except in pigeons, macaws, and conures because effective concentrations could not be achieved in these birds (Clark et al., 1982). However, oral absorption was poor, and this route of administration was discouraged for all birds.

Chloramphenicol Derivatives

The ban on the use chloramphenicol in food-producing animals in the mid-1980s left a gap in the veterinarian's armamentarium of effective antimicrobial drugs. Because the idiosyncratic aplastic anemia is associated with the presence of the para-nitro group on the chloramphenicol molecule, attempts were made to modify the chloramphenicol structure to simultaneously retain chloramphenicol's broad spectrum of antimicrobial activity and eliminate the induction of aplastic anemia in people. Compounds synthesized in attempts to accomplish this goal are thiamphenicol and florfenicol. Thiamfenicol is not approved in the United States and will be discussed here only briefly. However, florfenicol has been approved for use in pigs, cattle, and fish (in some countries) and has been effective for treatment of various infections, especially bovine respiratory disease in cattle and swine respiratory disease in pigs intended for human consumption.

Thiamphenicol

Thiamphenicol is a semisynthetic structural analog of chloramphenicol. It is not available in North America; therefore, all of the experience has been learned from research studies or use in other countries. The major structural difference between chloramphenicol and thiamphenicol is that the para-nitrophenol group has been replaced by the methyl sulfonyl moiety (Figure 36.1). The mechanism of action and spectrum are similar to that of chloramphenicol. However, its structural differences result in different pharmacokinetic properties and decreased potency. Thiamphenicol is more water soluble and less lipid soluble and therefore diffuses more slowly through lipid membranes. It is not metabolized to a significant extent in the liver (Ferrari et al., 1974) and most of the dose is excreted in the urine as the unchanged active compound (Yunis, 1988; Lavy et al., 1991a; Gamez et al., 1992). Resistance to thiamphenicol is also similar to that of chloramphenicol, with bacterial acetylation of the thiamphenicol molecule, but at a rate approximately 50% less than that of chloramphenicol.

Few pharmacokinetic studies have been performed on food-producing animals, but thiamphenicol pharmacokinetics has been studied in veal calves (Gamez et al., 1992) and lactating goats (Lavy et al., 1991a). Both studies found thiamphenicol to have a large volume of distribution and rapidly eliminated in the urine. In dogs, thiamfenicol had a half-life of 1.7 hours and a volume of distribution of 0.66 l/kg (Castells et al., 1998). In dogs the injection of thiamfenicol was well absorbed, with availability of 97%, but the terminal half-life was longer (5.6 hours), suggesting slow release from the injection site.

Thiamphenicol is considered to be less toxic than chloramphenicol, yet a reversible bone marrow suppression has been reported in humans. However, millions of people have been treated with thiamphenicol in countries in which it is approved, with no reports linking its use to aplastic anemia (Adams et al., 1987). In a thiamphenicol toxicity study in rabbits (Kaltwasser et al., 1974), no changes attributed to thiamphenicol in erythrocyte, reticulocyte, or plasma iron parameters were noted after long-term treatments of up to 90 mg/kg/day.

Florfenicol

Florfenicol is structurally related to thiamphenicol; however, florfenicol contains fluorine at the 3′ carbon position (Figure 36.1). The fluorine molecule substitution at this position also reduces the number of sites available for bacterial acetylation reactions to occur, possibly making the antibiotic more resistant to bacterial inactivation. Florfenicol is as potent, or more potent, than either chloramphenicol or thiamphenicol against many organisms in vitro. The study by Maaland et al. (2015) using *Staphylococcus pseudintermedius* and *E. coli* isolates, showed that there were fewer nonwild-type isolates for florfenicol than chloramphenicol. There were no nonwild-type isolates of *Staph. pseudintermedius* for florfenicol. These results agree with previous studies that show that resistance mechanisms may be less likely for florfenicol compared to chloramphenicol (Schwarz et al., 2004).

The list of susceptible bacteria for florfenicol is the same as listed previously for chloramphenicol. However, as mentioned earlier, some bacteria resistant to chloramphenicol because of inactivation by acetylation may be susceptible to florfenicol. The CLSI (CLSI, 2015) quality control ranges of MIC for florfenicol are 2–8 μg/ml (Marshall et al., 1996). *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* are several-fold more susceptible in vitro than bacteria of the Enterobacteriaceae, with MIC_{90} for *Pasteurella* and *Histophilus somni* in the range of 0.5–1.0 μg/ml. The CLSI breakpoints are ≤2 μg/ml (susceptible) 4 μg/ml (intermediate), and ≥8 μg/ml (resistant) for isolates of bovine and swine respiratory disease (CLSI, 2015). By comparison, the susceptible breakpoint for chloramphenicol is ≤8 μg/ml for organisms other than streptococci, and ≤4 μg/ml for *Streptococcus* spp. Florfenicol breakpoints for other animals and other bacteria have not been determined.

The advantage of florfenicol for administration to food animals is that it lacks the para-nitro group (Figure 36.1) that could contribute to the induction of aplastic anemia associated with chloramphenicol use in humans. Therefore, if residues were to occur in animals treated with florfenicol, no dangerous public health risk would ensue. However, it is possible that florfenicol can still produce a dose-related form of reversible bone marrow suppression with prolonged use or high doses. Such reactions have not been reported from routine use of florfenicol in animals, probably because it is rarely used for a long time. However, in one clinical account in a zoo animal, high doses induced bone marrow suppression (Tuttle et al., 2006).

Pharmacokinetics

The pharmacokinetics of florfenicol are summarized in Table 36.3.

Absorption: Studies in calves and other species listed in Table 36.3 show that absorption from all routes after either IM or SC injection is generally high. Oral absorption in horses and dogs was also high. After IM or SC injection, the absorption is slow and often prolonged in animals because of the vehicle in the solution. Therefore, the IM or SC injection produces a *flip-flop* effect, in which the terminal half-life is determined by the slow absorption. This can be seen in Table 36.3 in which the IM and SC half-life is generally much longer than the IV half-life. This effect prolongs the duration of effective concentrations.

Distribution: Like chloramphenicol, florfenicol has a wide distribution in most tissues of the body (Adams et al., 1987), with a volume of distribution approaching 1 l/kg (0.7–0.9 l/kg in most cattle studies) (Table 36.3). Protein binding is low in cattle with values of 13–19% reported (Bretzlaff et al., 1987; Lobell et al., 1994), but in other studies it was 5% at high concentrations and negligible at low concentrations in cattle plasma (Foster et al., 2016). Protein binding has not been reported for other animals. High concentrations are detected in the kidney,

Table 36.3 Selected pharmacokinetic parameters of florfenicol in animals

Species	Dose mg/kg	Half-life (h)	Absorption (%) (%)	Volume of distribution (l/kg)	C_{max} (μg/ml)	Reference
Cats	22 (all routes)	4 (IV)	–	0.61	57 (IV)	Papich, 1999
		7.8 (oral)	>100 (oral)	–	28 (oral)	
		5.6 (IM)	>100 (IM)	–	20 (IM)	
Dogs	20 mg (all routes)	2 (IV)	28 (SC)	1.2	44 (IV)	
		18 (SC)	16 (IM)	–	0.93 (SC)	
		9 (IM)	–	–	1.64 (IM)	
		3 (oral)	>100 (oral)	–	17 (oral)	
	20 IV	1.11	–	1.45	–	Park et al., 2008
	20 oral	1.24	95.43	–	6.18	Park et al., 2008
Sea turtles	20 IM, IV	2–7.8 h (IM)	67 (IM)	10–60	0.5–0.8 (IM)	Stamper et al., 2003
Sharks	40 IM	269	ND	2.9	10.5	Zimmerman et al., 2006
Fish (red pacu)	10 IM	4.25	ND	5.69	1.09	Lewbart et al., 2005
Horses	22 IV	1.83	81 (IM)	0.72	4 (IM)	McKellar and Varma, 1996
	22 oral	ND	83 (oral)	ND	13 (oral)	
Cattle	50 IV	3.2	ND	0.67	157.7	Bretzlaff et al., 1987
Feeder calves	20 IV	2.65	ND	0.88	73	Lobell et al., 1994
	20 IM	18.3	78.5	ND	3.07	Lobell et al., 1994
Veal calves	22 oral	ND	88	ND	11.3	Varma et al., 1986
	22 IV	2.87	ND	0.78	66	Varma et al., 1986
	11 IV	3.71	ND	0.91	26.35	Adams et al., 1987
	11 oral	3.7	89	ND	5.7	Adams et al., 1987
Angus calves	40 SC	27.5	ND	ND	6.04	Sidhu et al., 2014
Dairy calves	40 SC	28.44	ND	ND	3.42	Foster et al., 2016
Lactating cows	20 IV	2.9	–	0.35	12.4	Soback et al., 1995
	20 IM	12	38	ND	3.6	
Alpaca	20 IM	17.59	ND	11.07 (Vd/F)	4.31	Holmes et al., 2012
	40 SC	99.67	ND	55.74 (Vd/F)	1.95	
Llama	20 IV	2.2	63	0.96	–	Pentecost et al., 2013
	20 IM	11.6	–	–	3.2	
Camels	20 IV	1.49	69.2	0.89	–	Ali et al., 2003
	20 IM	2.52	–	–	0.84	
Sheep	20 IV	1.31	65.8	0.69	–	
	20 IM	2.28	–	–	1.04	
Goats	20 IV	1.19	60.9	0.57		
	20 IM	2.12	–	–	1.21	

Route of administration used is listed with dose. C_{max} is the maximal concentration after administration. ND, not determined. Empty cells indicate that the parameter is not relevant for the route administered.

urine, bile, and small intestine, but less penetration in the CSF, brain, and aqueous humor of the eye than that attained with chloramphenicol. Concentrations in brain and CSF are one-quarter and one half the corresponding concentrations in plasma, respectively. Although in one study the distribution into CSF was only 46% relative to plasma, these levels were high enough to produce concentrations in CSF of cattle to inhibit *Histophilus somni* for over 20 hours (De Craene et al., 1997). Florfenicol reached high concentrations in the synovial fluid of cattle following regional limb perfusion (Gilliam et al., 2008). Florfenicol also penetrated well into the milk of lactating goats after IV and IM administration; therefore, it could be used to treat microbial infections in the udder of lactating animals (Lavy et al., 1991b) if appropriate milk withdrawal times are available. The penetration into interstitial fluid was almost 98%, a reflection of the low protein binding. In the same study, the penetration into the pulmonary epithelial lining fluid of calves was over 200% and produced high concentrations for treating respiratory infections (Foster et al., 2016).

Metabolism and Elimination: The elimination half-life in various species and for different routes is shown in Table 36.3. Most of the dose administered to cattle is excreted as the parent drug (64%) in the urine, with the remaining excreted as urinary metabolites. Florfenicol amine is the metabolite that persists longest in tissues of cattle and is used as the marker residue for withdrawal determination.

Pharmacokinetic studies in other species: As seen in Table 36.3, there have been pharmacokinetic studies in small animals and some exotic and zoo animals. As in cattle, it has rapid clearance when injected IV, but more prolonged terminal half-life if administered by other routes.

Pharmacokinetic–pharmacodynamic properties: The Pharmacokinetic–pharmacodynamic (PK-PD) properties for florfenicol may be dependent on the organism studied. There is evidence for a bactericidal effect against some bacteria but not others (Maaland et al., 2015; Sidhu et al., 2014). Florfenicol may be bactericidal against isolates of *Staph. pseudintermedius but not E. coli* (Maaland et al., 2015). Against bovine isolates of *Mannheimia haemolytica* and Pasteurella multocida, florfenicol appears to have bactericidal activity. It is not established if the parameter for predicting efficacy is time above MIC (T>MIC) or area-under-the-curve (AUC) / MIC. It is likely that as for most other protein synthesis inhibiting agents with little or no postantibiotic effect, the AUC/MIC would be the best parameter to predict clinical efficacy. In a study in calves (Sidhu et al., 2014) a AUC/MIC ratio of approximately 18–27 was identified from modeling experiments.

Clinical Use

Florfenicol is available in three injectable solutions: 300 mg/ml solution for injection (Nuflor, or Nuflor gold), and a solution combined with flunixin meglumine (Resflor Gold, 300 mg/ml florfenicol plus 16.5 mg/ml flunixin). There is also a solution to be added to drinking water for swine (23 mg/ml, to be added as 400 mg per gallon) and a Type A medicated feed. For fish there is a 500 gram per kilogram premix for fish (Aqua-Flor).

Cattle and pigs: Several studies in cattle have been conducted to support the use of florfenicol for treating bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni*. Florfenicol penetrates well into the epithelial lining fluid of the airways of cattle (Foster et al., 2016), and has been effective for treating undifferentiated bovine respiratory disease (Hoar et al., 1998; Jim et al., 1999). There are two doses approved for cattle: 20 mg/kg SC or IM, given every 48 hours and injected in the neck, or a single dose for cattle at 40 mg/kg SC in the neck. Florfenicol is also approved for treatment of bovine interdigital phlegmon (foot rot, acute interdigital necrobacillosis, infectious pododermatitis) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*.

Florfenicol has been effective in calves for treating experimentally induced infections and naturally occurring infectious bovine keratoconjunctivitis (Dueger et al., 1999; Angelos et al., 2000). In the naturally occurring case, florfenicol was administered one dose SC at 40 mg/kg or IM two doses 48 hours apart at 20 mg/kg. Concentrations persist in CSF for a long enough period after administration of 20 mg/kg in cattle that concentrations are above MIC of susceptible pathogens for at least 20 hours.

The swine dose is 15 mg/kg IM in the neck, every 48 hours. For pigs, florfenicol can also be added to the feed (182 g per ton of feed), or drinking water (400 mg per gallon) for 5 days for the control of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Streptococcus suis,* and *Bordetella bronchiseptica*.

Small animals: Although some pharmacokinetic studies have been conducted in small animals and exotic animals, there are no reports of efficacy. Pharmacokinetic studies in reptiles and dogs suggest that frequent dosing with high doses would be necessary to maintain plasma concentrations above the MIC for susceptible organisms throughout the dosing interval. By contrast, florfenicol solution in cats was absorbed well from both routes, with peak concentrations of 20 μg/ml and 27 μg/ml after IM and oral dose, respectively. Absorption was high from both routes (greater than 100% from IM and oral). The half-life was 5.6 hours and 7.8 hours for IM and oral dose, respectively. In cats, florfenicol produced inhibitory concentrations for 12 hours. These studies indicate that a dose of 22 mg/kg administered every 12 hours orally or parenterally would be adequate to produce sustained plasma concentrations for treatment of susceptible bacteria. Safety of these doses for small animals have not been established.

Topical forms: Two topical formulations were approved by the FDA in 2014 and 2015. The product Osurnia® contains 10 mg florfenicol, 10 mg terbinafine, and 1 mg betamethasone acetate per ml in a gel for topical administration. The product Claro™ contains florfenicol 15 mg/ml, terbinafine 13.3 mg/ml, and mometasone 2 mg/ml. The indication for each product is for the treatment of otitis externa in dogs associated with susceptible strains of bacteria (*Staphylococcus pseudintermedius*) and yeast (*Malassezia pachydermatis*).

Use in fish: Florfenicol has been administered orally for treatment of infections in captive fish and is approved in some countries for this use (Aqua-Flor®). Florfenicol is effective for treating bacterial infections in fish, such as trout and salmon (Fukui et al., 1987). Florfenicol premix is approved in some countries for treatment of furunculosis in salmon caused by *Aeromonas salmonicida*. Florfenicol has been administered orally for treatment of furunculosis caused by susceptible strains of *Aeromonas* *salmonicida* in captive fish and is approved in other countries. The premix (Aqua-Flor®) is approved for use in catfish and salmonids at a dose of 10 mg/kg for 10 days to treat susceptible fish pathogens.

The typical dose for fish is 10 mg/kg. At this dose, the half-life is 12–16 hours in most fish, with a peak (C_{max}) concentration of approximately 3–10 μg/ml (Pinault et al., 1997; Martinsen et al., 1993; Horsberg et al., 1994). Red pacu had a shorter half-life and lower C_{max} after 10 mg/kg IM (Lewbart et al., 2005). In sharks at a dose of 40 mg/kg IM, florfenicol produced effective levels for 120 hours (Zimmerman et al., 2006). Admnistration to sharks every 3–5 days will produce concentrations in a therapeutic range.

Other species: Pharmacokinetic studies in horses show that florfenicol has longer half-life than chloramphenicol, good distribution, and good absorption. However, in experimental horses there were consistent loose stools and elevated bilirubin (McKellar and Varma, 1996). Until additional studies are available to establish safe doses, florfenicol cannot be recommended for horses. Adverse effects were observed in alpaca at repeated doses of 40 mg/kg SC, but not in llamas administered a single dose of 20 mg/kg (Holmes et al., 2012; Pentecost et al., 2013). The authors recommended a dose in llamas of 20 mg/kg once daily, IM. After a single injection of 20 mg/kg (IM and IV) there were no adverse effects identified clinically or in blood tests in camels, sheep, or goats (Ali et al., 2003). However, the low concentrations achieved and the short half-life in the study by Ali et al., raises questions about whether or not it would be clinically effective at this dose.

In snakes (boas), the half-life was 28 hours from IM injection. It was estimated that 50 mg/kg once daily for boas is the best dose to produce therapeutic plasma concentrations, even though efficacy studies are not available. In sea turtles the clearance was rapid (60–100 ml/kg/h) and the half-life was short (Stamper et al., 2003). It was concluded that florfenicol was not a practical drug for treatment of infections in sea turtles.

Adverse Effects

Effect of florfenicol on bovine pregnancy, reproduction, and lactation have not been determined. Mild diarrhea and elevated bilirubin have been reported from administration to horses (McKellar and Varma, 1996). Reversible, dose-related bone marrow suppression is possible but not reported, except for a reaction reported in a zoo animal that was mentioned previously (Tuttle et al., 2006). In cattle, diarrhea and decreased feed consumption have been observed, which are transient. A local tissue reaction from IM or SC injection is possible. When toxic overdoses were administered to calves (200 mg/kg) there was marked anorexia, decrease in body weight, ketosis, and elevated liver enzymes. In dogs administered high doses for prolonged periods there was CNS vacuolation, hematopoietic toxicity, and renal tubule dilation. Adverse effects were detected in alpacas after a dose of 40 mg/kg SC that may be related to the prolonged concentrations at this dose (Table 36.3) (Holmes et al., 2012). These effects included significant hematological abnormalities and protein decrease. Caution should be used if administering to these animals for repeated doses.

Regulatory Information

The tolerance for florfenicol is 3.7 ppm for florfenicol amine (the marker residue) in liver and 0.3 ppm in muscle. Withdrawal time for use in salmon is 12 days. Withdrawal time for oral administration to pigs in feed is 13 days and for administration in water 16 days. After injection to cattle, the withdrawal time for slaughter is 28 days if injected at a dose of 20 mg/kg IM (36 days in Canada). If injected at a dose of 40 mg/kg SC, the withdrawal time for slaughter is 38 days. A formulation with different excipients (Nuflor Gold) has a withdrawal time of 44 days in cattle when injected at 40 mg/kg SC, once. More than 10 ml should not be injected at each site to avoid tissue reactions and injections should be administered in the neck (both SC and IM). Do not administer to dairy cows older than 20 months, to calves under 1 month of age, or to calves on an all-milk diet.

Macrolide Antibiotics

Source and Chemistry

The macrolide antibiotics are a group of structurally similar compounds, most of which are derived from various species of *Streptomyces* soil-borne bacteria. Chemically, all the drugs in this group are classified as macrocyclic lactones, with members containing 12–20 atoms of carbon in the lactone ring structure (Table 36.4). Attached to this lactone ring are various combinations of deoxy sugars held to the lactone ring

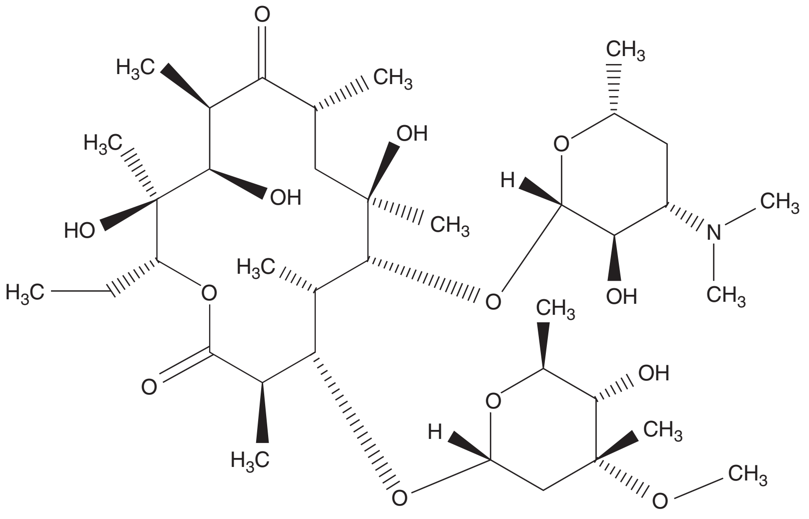
Table 36.4 Macrolides used in animals

Drug	Structure	Brand Name
Erythromycin	14 member ring	Gallimycin (and generic)
Tilmicosin	16 member ring	Micotil, Pulmotil
Azithromycin	15 member ring	Zithromax (human drug)
Gamithromycin	15 member ring	Zactran
Tylosin	16 member ring	Tylan
Tildipirosin	16 member ring	Zuprevo
Tulathromycin	15 member ring	Draxxin

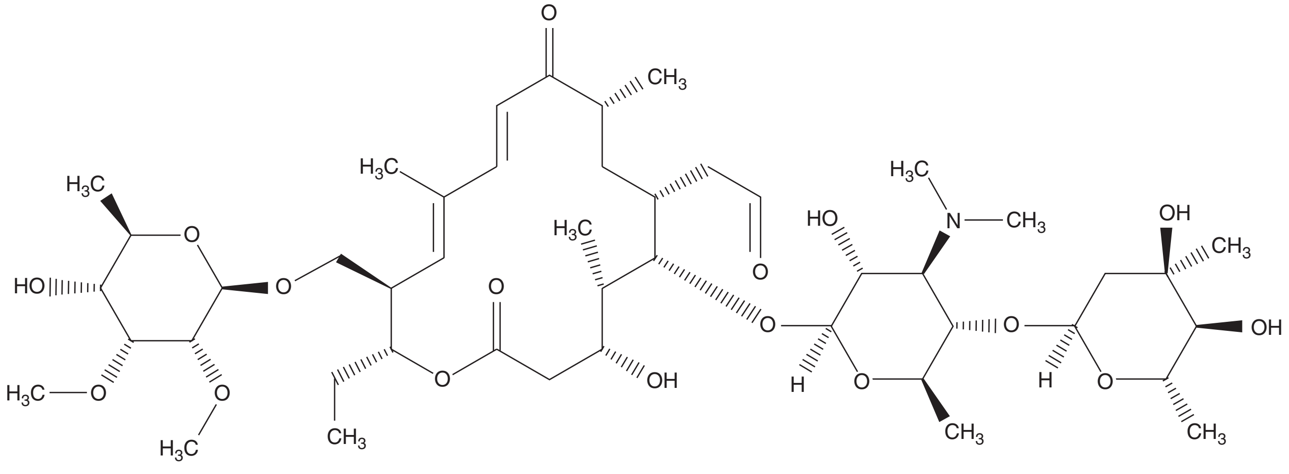
by glycosidic linkages. Since erythromycin's discovery in the early 1950s from the soil organism *Streptomyces erythreus,* numerous other macrolides have been isolated or synthesized from the parent molecule erythromycin, including tylosin, roxithromycin, erythromycylamine, tilmicosin, dirithromycin, azithromycin, tulathromycin, clarithromycin, spiramycin, and flurithromycin (Kirst and Sides, 1989). The most common agents used clinically in veterinary medicine are listed in Table 36.4.

Erythromycin and tylosin (Figure 36.2) are traditional macrolides. The newer drugs (Table 36.4 and Figure 36.3) were either developed for use in people (azithromycin) or specifically for use in cattle and/or pigs. These newer drugs differ from erythromycin in that they have a prolonged action and can be administered intermittently, or for just a single injection. Other macrolides such as oleandomycin and carbomycin have been used as feed additives for growth promotion in food animals and will not be discussed in detail here.

Macrolides are composed of a macrolactone ring of 12, 14, 15, or 16 carbon atoms, substituted with sugar moieties. Erythromycin, the prototype of this class, consists of a 14-atom polyhydroxylactone erythronolide ring and the two sugars clandinose and desosamine. Similarly, tylosin is composed of a 16-atom lactone ring (a tylonolide) to which three sugars – mycinose, mycaminose, and mycarose – are attached (Wilson, 1984; Kirst et al., 1982). Azithromycin is the first drug in the group of azalides, which are semisynthetic derivatives of erythromycin (Lode et al., 1996). Azithromycin has a 15-member ring structure. Tulathromycin, resembles azithromycin (Figure 36.3) and also has a 15-member ring structure. The structure of the newer agents includes basic nitrogen groups. All macrolides are weak bases, with pK_a ranges



Erithromycin



Tylosin

Figure 36.2 The chemical structures of erythromycin (top) and tylosin (bottom).

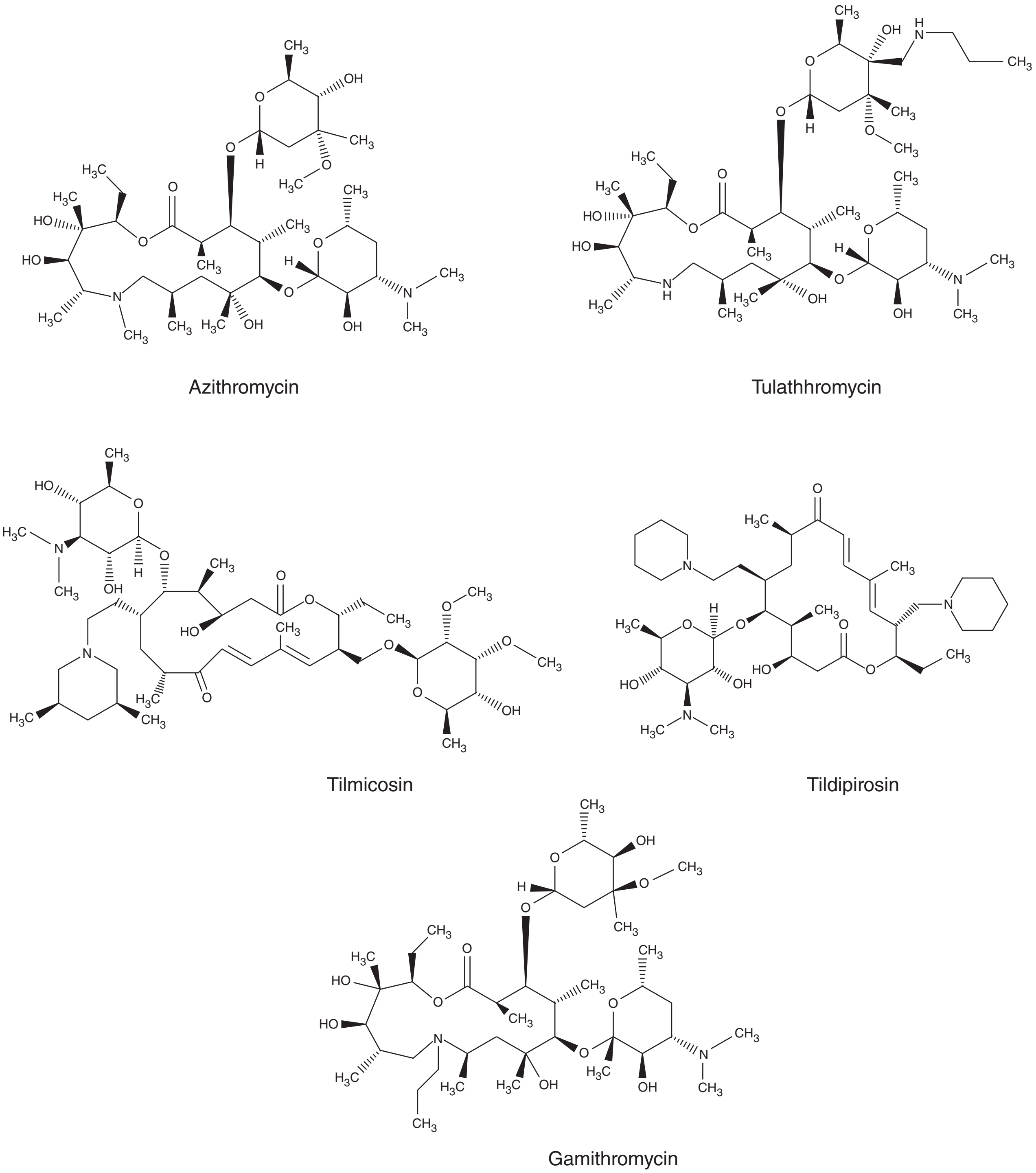


Figure 36.3 The chemical structures of new macrolide derivatives: azithromycin, tulathromycin, tildipirosin, tilmicosin, and gamithromycin. Azithromycin is a human drug and the others are approved for use in cattle and/or pigs.

from 6 to 9. They can have either two (di-basic) or three (tri-basic) nitrogen groups. For example, tulathromycin has three (tribasic) and has been referred to as a "trimilide". Tildipirosin also has three basic groups. The basic nitrogen groups on these newer agents (Figure 36.3) produces a positive charge in an acidic environment below their pKa. The positive charge increases the affinity for intracellular sites caused by ion trapping. It is this property that gives these agents such large intracellular distribution and a high volume of distribution.

Effect on Antibacterial Activity: The basic nature of the macrolides also influences the antibacterial activity. The in vitro antibacterial activity of macrolides varies according to the pH of the culture medium and the pH at the site of infection. Subsequently, antibacterial activity decreases in acid pH and increases in alkaline conditions. A change in pH of only 0.2 units has been known to change the MIC by a full Log-2 dilution step. These changes become important when CO_2 is used during culture incubation because it lowers the pH of the medium.

Mechanism of Action

The antibacterial action of macrolides is produced by an inhibition of protein synthesis by binding to the 50S ribosomal subunit at the 23S rRNA site of prokaryote organisms. The binding site on the ribosome is near, but not identical to, that of chloramphenicol, and antagonism of effect is possible if macrolides are administered with chloramphenicol. By binding to the 50S ribosomal site, macrolides cause dissociation of peptidyl-tRNA from the ribosomes during the elongation phase, which disrupts addition of new peptide bonds and thus prevents synthesis of new proteins. Although macrolides can bind to mitochondrial ribosomes, they are unable to cross the mitochondrial membrane (in contrast to chloramphenicol) and do not produce bone marrow suppression in mammals. Macrolides do not bind to mammalian ribosomes, making them a relatively safe group of drugs for veterinary use.

Although most authors have listed macrolides as bacteriostatic at therapeutic concentrations (Wilson, 1984), this effect may be both bacterial species, concentration, and drug dependent. For example, the agents developed for pigs and cattle (Table 36.4) can have bactericidal activity against bovine and swine respiratory pathogens, including *Mannheimia haemolytica, Pasteurella multocida*, *Histophilus somni,* and *Actinobacillus pleuropneumoniae*. As discussed above, the antimicrobial action of macrolides is enhanced by a high pH (Sabath et al., 1968), with the optimum antibacterial effect at a pH of 8. Therefore, in an acidic environment, such as in an abscess, necrotic tissue, or urine, the antibacterial activity is suppressed.

Resistance Mechanisms

Resistance to macrolides is usually plasmid mediated, but modification of ribosomes may occur through chromosomal mutation. Resistance can occur from: (i) decreased entry into bacteria (most common with the gram-negative organisms), and also mediated by *mef*-efflux genes, (ii) synthesis of bacterial enzymes that hydrolyze the drug, and (iii) modification of target (the ribosome in this instance) by RNA methylation or RNA sequence changes through mutation. The ribosomal attenuation (most common mechanism) involves methylation of the 50S drug receptor site. This resistance, coded by *erm* genes (e.g., *erm*A, *erm*B, *erm*C) may also lead to cross-resistance with other antibiotics that preferentially bind to these sites, such as other macrolides and lincosamides (Wilson, 1984). Resistance to erythromycin in animals in several microorganisms has been discussed in more detail elsewhere (Maguire et al., 1989; Dutta and Devriese, 1981, 1982a, 1982b; Leclercq and Courvalin, 1991; Devriese and Dutta, 1984). In small animals with staphylococcal infections, resistance was more likely if antibiotics had previously been prescribed, especially in cases of recurrent pyoderma (Lloyd et al., 1996; Medleau et al., 1986; Noble and Kent, 1992). Seven to 22% of small animal isolates of *Staphylococcus* spp. can exhibit resistance (depending on region and use), and in some countries this has remained relatively stable at around 22–24%.

Spectrum of Activity

Erythromycin is mainly effective against gram-positive organisms such as streptococci, staphylococci, including staphylococci that may be resistant to β-lactams because of β-lactamase synthesis or modification of the penicillin-binding protein target. Other organisms that show in vitro susceptibility to macrolides include *Mycoplasma, Arcanobacterium, Erysipelothrix, Bordetella,* and *Bartonella*. Although the spectrum favors the gram-positive group, a few gram-negative bacteria are susceptible, especially *Pasteurella* spp. Activity against anaerobic bacteria is only moderate. Most other gram-negative bacteria, such as those of the Enterobacteriaceae or *Pseudomonas* spp., are resistant. Azithromycin is an exception among the macrolides and can exhibit more activity against gram-negative bacteria. In addition to better activity against Enterobacteriaceae, it also has activity against other enteric pathogens, such as *Campylobacter* spp. (Gordillo et al., 1993).

The activity of newer derivatives (Table 36.4 and Figure 36.3) is similar to that of erythromycin, but these agents have better activity against respiratory pathogens, including *Pasteurella, Mannheimia haemolytica,* and *Histophilus somni,* which corresponds to their use for treating respiratory tract infections in pigs and cattle. These macrolides also have activity against *Mycoplasma* spp. The activity of macrolides against *Rhodococus equi* is important for treating lung infections caused by this organism in horses, particularly foals (Jacks et al., 2003). The MIC values for 32 antimicrobials against *Rhodococcus equi* were compared by Riesenberg et al. (2014). In decreasing order of activity, the MIC_{90} values for clarithromycin, erythromycin, azithromycin, tilmicosin, tylosin, tulathromycin were 0.06, 0.5, 1, 32, 32, and 64 μg/ml, respectively. In a separate study,

Table 36.5 Interpretive criteria for macrolides and lincosamides used in animals. Source: Data from CLSI, 2015.

		MIC Interpretive Category (µg/ml)			
Drug	**Species**	**S**	**I**	**R**	**Comments / Pathogens**
Erythromycin	Humans	≤ 0.5	1-4	≥ 8	Human *Staphylococcus*. No criteria available for animals.
	Humans	≤ 0.25	0.5	≥ 1	Human *Streptococcus*. No criteria available for animals.
Azithromycin	Humans	≤ 0.5	1	≥ 2	Human only. No criteria available for animals.
Tilmicosin	Bovine	≤ 8	16	≥ 32	Bovine respiratory pathogens.
	Swine	≤ 16	–	≥ 32	Swine respiratory pathogens
Tulathromycin	Bovine	≤ 16	32	≥ 64	Bovine respiratory pathogens (*Mannheimia, Pasteurella,* Histophilus)
	Swine	≤ 16	32	≥ 64	*Pasteurella multocida, Bordetella bronchiseptica*
		≤ 64	–	–	*Actinobacillus pleuropneumoniae*
Tildipirosin	Bovine	≤ 8	16	≥ 32	Bovine respiratory pathogens (*Histophilus, Pasteurella*)
		≤ 4	8	≥ 16	Bovine respiratory pathogens (*Mannheimia*)
	Swine	≤ 8	–	–	*Bordetella bronchiseptica*
		≤ 4	–	–	*Pasteurella multocida*
		≤ 16	–	–	*Actinobacillus pleuropneumoniae*
Gamithromycin	Bovine	≤ 4	8	≥ 16	Bovine respiratory pathogens. (*Mannheimia, Pasteurella*, Histophilus)
Clindamycin	Canine	≤ 0.5	1-2	≥ 4	*Staphylococcus* spp. *Streptococcus* spp.

S, susceptible; R, resistant; I, intermediate.

gamithromycin had a MIC_{90} of 1 µg/ml (Berghaus et al., 2012). Therefore, all macrolides are not alike with respect to their activity against this equine pathogen.

The CLSI (CLSI, 2015) standards and interpretive categories are shown in Table 36.5 for susceptibility testing. As shown in this table, drugs in this class vary in their potency and activity against various pathogens. Because of their targeted use, most of this data were generated for respiratory pathogens (Watts, 1999).

Pharmacokinetic–Pharmacodynamic Properties

The PK/PD properties of macrolides have been more difficult to define compared to other antimicrobials. Plasma concentrations, especially for the newer, long-acting agents (Table 36.6) are often below the MIC of pathogens for most, or all of the dose interval. Therefore, parameters such as peak above MIC (C_{max}/MC) or time above MIC (T>MIC) cannot be used to predict efficacy. Efficacy is probably best attributed to the concentrations at the site of infection – the pulmonary epithelial lining fluid (PELF). Although concentrations in the PELF have been reported from many studies in research animals (Guigère and Tessman, 2011; Villarino et al., 2013), this fluid is difficult to sample routinely in clinical cases. Therefore, the plasma drug concentration has been examined as a surrogate marker for efficacy from administration of macrolides and their derivatives. The parameter that is best suited to predict efficacy is the AUC of the plasma drug concentration to MIC (AUC/MIC) ratio (Drusano, 2005; Toutain et al., 2017). It has been suggested that the high concentrations in inflammatory cells deliver high concentrations to infected site and this effect is responsible for the efficacy in infected tissues. However, as summarized in the review by Villarino et al. (2013), citing studies by their laboratory and others, the concentrations contained in these cells are not likely high enough to contribute significantly to the PK/PD properties of the macrolides. This view was supported by the analysis by Toutain and associates (Toutain et al., 2017).

The magnitude of the AUC/MIC target has emerged from laboratory animal studies and analysis of clinical results. The azithromycin free serum AUC/ MIC for a 24-hour interval (AUC_{24}) ratio of >25 has been suggested from a mouse thigh infection model reported by Craig et al. (2002). However, this ratio is likely lower in non-neutropenic animals and Rodvold et al. (2003) suggested plasma AUC_{24}/ MIC ratios of at least 10 in nonneutropenic hosts with pneumonia, and higher AUC_{24}/ MIC ratios of 25–30 for worst case scenarios with experimental neutropenia. The study by Sevillano et al. (2006) showed that a serum azithromycin AUC_{24}/MIC ratio of approximately 27 was adequate for sustained bactericidal activity against susceptible strains. The analysis by Toutain and colleagues cited earlier supported a AUC_{24}/MIC value of approximately 24 for tulathromycin treatment of pneumonia in calves (Toutain et al., 2017).

Because most of the newer macrolides have long half-lives and produce concentrations for much longer than 24 hours, some investigators have considered the AUC values for the duration of treatment, rather than limited to 24 hours. In the study by Muto et al. (2011), using this approach, the AUC/MIC ratio >5 for azithromycin was associated with successful clinical outcome. If one examines the pharmacokinetics from studies of the

Table 36.6 Pharmacokinetics of macrolide derivatives, including azalides, in animals

Drug	Species	Dose (mg/kg)	Half-life (hour)[a]	Volume of distribution (Vd) (l/kg)[b]	Peak Concentration (C_{max}) (μg/ml)	Reference
Azithromycin	Foals	10 IV	20.3	18.6	–	Jacks et al., 2003
	Foals	10 oral	44 (MRT)	–	0.57	
	Foals	5 IV	16	12.4	–	Davis et al., 2002
	Foals	10 oral	18.32	–	0.72	
	Dogs	20 oral	35	–	4.2	Shepard and Falkner, 1990
	Dogs	20 IV	29	12	–	
	Cats	5 IV	35	23	–	Hunter et al., 1995
	Cats	10 oral	30 (MRT)	–	0.97	
Clarithromycin	Foals	10 oral	4.81	–	0.92	Jacks et al., 2002
	Foals	7.5 IV	5.4	10.4	–	Womble et al., 2006
	Foals	7.5 oral	7.1 (MRT)	–	0.52	
	Foals[c]	7.5 oral	6.11	–	0.614	Peters et al., 2011
	Foals[c]	7.5 oral	7.17	–	0.61	Peters et al., 2012
	Foals[c]	7.5 oral	5.62	–	0.27	Berlin et al., 2016
	Foals[c]	7.5 IV	5.91	–	1.71	Berlin et al., 2016
	Dogs	10 IV	3.9	1.4	–	Vilmànyi et al., 1996
	Dogs	10 (oral tablet)	4.6-5.9	–	3.3-3.5	
Gamithromycin	Calves	6 SC	62	–	0.43	Giguére et al., 2011
	Calves	6 SC	50.8	24.9	0.75	Huang et al., 2010
	Feeder cattle	6 SC	52.8 (MRT)	97.4 (V/F)	0.13	DeDonder et al., 2016
	Foals	6 IM	39.1	–	0.33	Berghaus et al., 2012
	Sheep	6 SC	34.5	35.5 (V/F)	0.573	Kellermann et al., 2014
Tildipirosin	Calves	4 SC	210	–	0.71	Menge et al., 2012
	Calves	6 IV	204	49.4	0.64	
	Pigs	4 IM	106	–	0.895	Rose et al., 2013
Tulathromycin	Calves	2.5 SC	81.24	–	1.82	Foster et al., 2016
	Calves	2.5	79.5	11	0.39	Villarino et al., 2013[d]
	Pigs	2.5	73.95	28.9	0.75	
	Horses	2.5	122	–	0.57	
	Goats	2.5	76.7	29.3	0.94	
	Goats	2.5 SC	45.7	7.0 (V/F)	1.0	Romanet et al., 2012
	Sheep	2.5 SC	118.4	–	3.6	Washburn et al., 2014
Tilmicosin	Calves	20 SC	33.34	5.5 (V/F)	3.48	Foster et al., 2016
	Dairy cow	10 IV bolus	0.76	2.14	–	Ziv et al., 1995
	Dairy cow	10 SC	4.18	–	0.13	
	Beef cattle	10 IV	28	28.2	1.56	Lombardi et al., 2011
	Beef cattle, light	10 SC	31.15	–	0.71	
	Beef cattle, light	20 SC	31.13	–	1.06	
	Beef cattle, heavy	10 SC	30.83	–	0.55	
	Beef cattle, heavy	20 SC	30.98	–	1.07	
	Pigs	20 oral	25.3	–	1.19	Shen et al., 2005
	Pigs	40 oral	20.7	–	2.03	

[a] For some studies, half-life was not reported and mean residence time (MRT) is listed in the table.

[b] For some studies, the volume of distribution was from a nonintravenous route and is listed as Vd/F.

[c] Data listed for clarithromycin by Peters et al., (2011, 2012), and Berlin et al., (2016) is without coadministration of rifampin. Administration of rifampin with clarithromycin lowers the concentration by 70% to over 90%. These values are provided in detail in those papers.

[d] Data referenced for Villarino et al. (2013) are an average of multiple studies reported in their paper.

long-acting macrolides listed in Table 36.6, AUC/MIC ratios of 5–10 for gamithromycin, azithromycin, and tildipirosin have been associated with clinical success. Tildipirosin, which has a longer half-life, produces a ratio of approximately 24. The study by DeDonder et al. (2016) showed that for gamithromycin administration to feeder cattle with bovine respiratory disease associated with *Mannheimia haemolytica* or *Pasteurella multocida*, the AUC infinity/MIC ratio associated with clinical success in these cases was 3.49 (*Mannheimia haemolytica*) and 3.21 (*Pasteurella multocida*).

Immunomodulatory Effects

Virulence properties of some bacteria may be inhibited by macrolides at concentrations that are less than the MIC required for inhibition or killing. This property, along with the effects on immunomodulation described in more detail below, may explain many of the benefits of macrolides for treating pneumonia (Kovaleva et al., 2012).

The macrolides, particularly the ones that concentrate in immune cells (Figure 36.3) have multiple immunomodulatory effects that likely contribute to the therapeutic response in respiratory infections, and perhaps other diseases. Beneficial effects may be produced by enhanced degranulation and apoptosis of neutrophils and inhibition of inflammatory cytokine production. Enhanced macrophage functions may also may help clear infections faster. These properties have been studied for azithromycin (Parnham et al., 2014) and for the veterinary drugs tilmicosin and tulathromycin (Chin et al., 2000; Duquette et al., 2015). As these reviews and studies point out, there is likely an immunomodulatory effect of these agents that contributes to the therapeutic benefits that is independent of the direct effect on bacteria. These drugs have been known to produce therapeutic benefits in patients even when the bacteria have MIC values in the range that is considered resistant, and above achievable concentrations in plasma or the epithelial lining fluid of the respiratory tract. The authors of these studies are careful to point out that the effect of macrolides is best termed *immunomodulatory* rather than immunosuppressive, which implies that it may modify or regulate functions of the immune system without impairing normal responses to combat bacterial infection (Kanoh and Rubin, 2010). According to Kanoh and Rubin (2010) the 14- and 15- membered macrolides exert these immunomodulatory effects, but not 16-membered macrolides. However, tilmicosin, a 16-membered ring macrolide (Figure 36.3, Table 36.4) also exhibits some of these properties (Chin et al., 2000).

As reviewed by others (Parnham et al., 2014; Kanoh and Rubin, 2010; Giamarellos-Bourboulis, 2008) macrolide antibiotics have shown inhibition in models of inflammation. These mechanisms include inhibition of inflammatory cells, improved epithelial function, and attenuated expression of inflammatory mediators. These properties have led to the recommended use of macrolides to treat some inflammatory diseases in people (Giamarellos-Bourboulis, 2008; Kanoh and Rubin, 2010). Because macrolides attain high concentrations in leukocytes and remain for a long time, primarily in lysosomes, there may be a biphasic response whereby initially the macrolides activate neutrophils and produce an initial burst that increases antibacterial activity, followed by suppression of inflammatory mediators and increased neutrophil apoptosis.

Pharmacokinetics

Absorption and Distribution

Erythromycin pharmacokinetics has been studied in most animals and in humans; some of these parameters are shown in Table 36.7. Tylosin pharmacokinetics in some animals is shown in Table 36.8. Oral erythromycin is discussed in more detail below in the section on erythromycin and summarized in Table 36.7. Tylosin has good absorption from the gastrointestinal tract, and no enteric coating is required to maintain the stability of the compound in the stomach. It is widely distributed to basically the same tissues as described for erythromycin, metabolized by the liver, and excreted via the bile and feces.

Oral absorption of most of the newer macrolides (Table 36.4) is not an issue because these are injected. For the others, oral absorption is moderate, but depends on the species. Azithromycin was absorbed 56% (Jacks et al., 2003) or 39% (Davis et al., 2002) in foals. It was absorbed 52% in cats (Hunter et al., 1995) and 97% in dogs (Shepard and Falkner, 1990). Clarithromycin was absorbed 57% in foals (Womble et al., 2006) and 71% in foals (Vilmànyi et al., 1996).

SC or IM injections of erythromycin can be painful and irritating; therefore, the PO route is preferred whenever possible. The only formulations that can be given IV are the glucoptate and lactobionate forms, because these are the only forms soluble in aqueous solution.

Pharmacokinetics of the newer macrolides are shown in Table 36.6. These properties have been extensively studied in many domestic animals. They have also been examined in several exotic and zoo animals (not shown in the table). These macrolides are characterized by much longer terminal half-lives compared to erythromycin. These long half-lives allow for intermittent administration (for example every-other-day in foals), and a single administration for gamithromycin, tildipirosin, tilmicosin, and tulathromycin in pigs and cattle. The volumes of distribution are very large, often in excess of 10 l/kg, and as high as 49 l/kg. The high volume of distribution is attributed to the extensive distribution to intracellular sites in tissues. Many of the studies referenced in Table 36.6 also reported tissue concentrations.

Macrolides tend to concentrate in some cells because the basic drug is trapped in cells that are more acidic than plasma. Tissue concentrations for macrolides, especially the newer azalides (Figure 36.3) are higher than serum concentrations. High concentrations have been documented in the respiratory tract, where PELF, bronchoalveolar fluid (BAL), leukocycte, and alveolar macrophages are many fold higher than plasma drug concentrations, often exceeding 100 times plasma concentrations. It is likely that the high concentrations in the epithelial lining fluid of the airways contribute significantly to the clinical efficacy for preventing and treating pneumonia.

Protein binding for macrolides is low to moderate, with values of 18–30% for most species. Protein binding in

Table 36.7 Selected serum pharmacokinetic parameters of erythromycin in animals

Species	Dose (mg/kg)	Route	Formulation	Half-life (hour)	Volume of distribution (l/kg)	Reference
Cows	12.5	IV	Base	3.16	0.789	Baggot and Gingerich, 1976
Calves	15	IV	Base in PG vehicle	2.91	0.835	Burrows et al., 1989
	15	IM	Base in PG vehicle	5.81	NA	Burrows et al., 1989
	15	SC	Base in PG vehicle	26.87	NA	Burrows et al., 1989
	30	IV	Base in PG vehicle	4.09	1.596	Burrows et al., 1989
	30	IM	Base in PG vehicle	11.85	NA	Burrows et al., 1989
	30	SC	Base in PG vehicle	18.3	NA	Burrows et al., 1989
Mice	10	IV	Base	0.65	3.6	Duthu, 1985
Rats	25	IV	Base	1.27	9.3	Duthu, 1985
Rabbits	10	IV	Base	1.4	6.8	Duthu, 1985
Dogs	10	IV	Base	1.72	2.7	Duthu, 1985
Dogs	25	Oral	Extolate	2.92	ND	Albarellos et al., 2008
Dogs	10	IV	Lactobionate	1.35	4.8	Albarellos et al., 2008
Pigs (1 day)	10	IV	Base	3.0	0.68	Kinoshita et al., 1995
Pigs (3 day)	10	IV	Base	1.43	3.28	Kinoshita et al., 1995
Foal	25	Oral	Ethylsuccinate	1.52	ND	Lakritz et al., 2002
	25	Oral	Base	1.8, 1.3	ND	Lakritz et al., 2000a
	25	Oral	Estolate	0.52	ND	Lakritz et al., 2000b
	25	Oral	Phosphate	0.81	ND	Lakritz et al., 2000b
	10	IV	Lactiobionate	1.18	0.91	Lakritz et al., 2000a
	10	IV	Lactiobionate	0.97	3.52	Lakritz et al., 1999
	25	Oral	Base	17.6 (MRT)	ND	Lakritz et al., 1999
	5	IV	Gluceptate	1.0	3.7	Prescott et al., 1983
	20	IV	Gluceptate	1.1	7.2	Prescott et al., 1983
Horse (Mares)	5	IV	Gluceptate	1.0	2.3	Prescott et al., 1983
Horse	25	Oral	Estolate	2.42	ND	Ewing et al., 1994
	37.5	Oral	Estolate	6.2	ND	Ewing et al., 1994
	25	Oral	Phosphate	2.49	ND	Ewing et al., 1994
	37.5	Oral	Phosphate	1.68	ND	Ewing et al., 1994
	25	Oral	Stearate	1.84	ND	Ewing et al., 1994
	25	Oral	Ethylsuccinate	4.76	ND	Ewing et al., 1994
Cats	15	PO	Ethylsuccinate	Not detectable concentrations		Albarellos et al., 2011
Cats	4	IV	Lactobionate	0.75	2.34	Albarellos et al., 2011

NA, data not available; PG, propylene glycol, MRT, mean residence time.

Table 36.8 Selected serum pharmacokinetic parameters of tylosin in animals

Species	Dose (mg/kg)	Route	Half-life ($t_{1/2\beta}$) (hour)	Vd (l/kg)	Reference
Dogs (Beagle)	10	IV	0.9	1.7	Weisel et al., 1977
Ewes	20	IV	2.05	NA	Ziv and Sulman, 1973b
Goats	15	IV	3.04	1.7	Atef et al., 1991b
Cows	12.5	IV	1.62	1.1	Gingerich et al., 1977
Cows	20	IV	2.14	NA	Gingerich et al., 1977
Calves					
2 days old	10	IV	2.32	7	Burrows et al., 1983
1 week old	10	IV	1.26	7.2	Burrows et al., 1983
2 week old	10	IV	0.95	11.1	Burrows et al., 1983
4 week old	10	IV	1.53	9	Burrows et al., 1983
>6 week old	10	IV	1.07	11.1	Burrows et al., 1983
Avians (emus)	15	IV	4.7	NA	Locke et al., 1982
Avians (quail, pigeons, cranes)	15	IM	1.2	NA	Locke et al., 1982

NA, data not available.

some species may be predominantly to the α-1-acid glycoprotein, rather than albumin (Kinoshita et al., 1995).

Metabolism and Excretion

Metabolism of erythromycin is by hepatic microsomal enzymes. For the other drugs, the metabolic pathway has been less well characterized. In people, most of azithromycin is excreted in the feces. Low concentrations are anticipated in the urine and kidney dysfunction is not expected to produce an appreciable effect on the elimination half-life in the body. Because of the low urine concentrations, lower activity at acidic pH, and spectrum of activity that does not favor Enterobacteriaceae, these agents are not a good choice for treating urinary tract infections (Sabath et al., 1968).

Adverse Effects and Precautions

When humans are treated with erythromycin, many adverse effects are reported, which include nausea and vomiting (oral forms), fever, skin eruptions, cholestatic hepatitis, elevated serum aspartate aminotransferase, epigastric distress, and transient auditory impairment, among many other side effects. Cholestatic hepatitis is associated with the estolate ester, with the symptoms starting 10–20 days after beginning therapy and ending a few days after the cessation of therapy. Cholestasis associated with erythromycin use in humans is considered to be a hypersensitivity reaction (Sande and Mandell, 1990a). In animals, these effects are less common. However, regurgitation and/or vomiting has been commonly reported in small animals, especially dogs after oral administration of erythromycin. In one report, erythromycin was the drug that most frequently caused side effects after oral dosing in dogs (Kunkle et al., 1995). Stimulation of gastrointestinal motility may play a role in small-animal vomiting (discussed in Section Clinical Use of Erythromycin to Modify Gastrointestinal Motility). Erythromycin has been associated with producing diarrhea in horses (Papich, 2003). Although these reactions in the horse may limit its use in some patients, it is still frequently used to treat infections in horses, especially in the foal. Hyperthermia (febrile syndrome) has been observed in foals associated with erythromycin treatment (Stratton-Phelps et al., 2000), which was accompanied in some foals by diarrhea and respiratory distress. Other adverse effects are discussed for specific agents in each section.

Drug Interactions

Erythromycin is a well-known hepatic microsomal enzyme inhibitor. Erythromycin is both a substrate and an inhibitor for the cytochrome P450 enzymes, which is the enzyme system that is most often involved in drug metabolism. As an inhibitor of the cytochrome P450 enzymes, it may inhibit metabolism of drugs such as theophylline, cyclosporine, digoxin, and warfarin. Concentrations of these drugs may increase when animals receive erythromycin, resulting in a potentiation of the pharmacological effect or toxicity.

Effects of the other macrolides on animal drug metabolism has not been investigated in much detail. Azithromycin can also be an enzyme inhibitor in people, but less so than for erythromycin. Nevertheless, concurrent use of any of the drugs in this class with other drugs that have a narrow therapeutic index should be monitored.

Erythromycin

Erythromycin is inactivated in the stomach due to gastric acidity, which is the reason that other formulations, such as erythromycin estolate or stearate forms or enteric-coated formulations, are used. These modified forms have better bioavailability owing to decreased destruction of erythromycin in the acidic environment of the stomach. Crushed tablets of enteric-coated preparations are substantially degraded in the stomach or are metabolized in the intestine wall and are not recommended for oral administration to animals. The presence of food in the stomach also tends to decrease absorption of erythromycin in most species, including the dog (Wilson, 1984; Eriksson et al., 1990). Erythromycin salts (erythromycin-stearate and erythromycin-phosphate) dissociate in the intestine and are absorbed as the active drug. Erythromycin esters (erythromycin-ethylsuccinate and erythromycin-estolate) are absorbed as the esters and hydrolyzed in the body to release active drug. There is no proven difference among these formulations as to which is the most favorable in most animals. However, in horses, it was shown that the salt forms (erythromycin phosphate or erythromycin stearate) are preferred for oral administration (Ewing et al., 1994) because they provided the most favorable blood concentrations. A series of studies by Lakritz et al. (1999, 2000a, 2000b, 2002) examined the absorption of various oral formulations in foals. The ethylsuccinate form was poorly absorbed, but the phosphate, estolate, and microencapsulated forms were better absorbed (16%, 14.7%, and 26%, respectively). Absorption was better in foals when food was withheld.

There are also oral formulations intended to be added to the feed or drinking water to treat infections for poultry. Examples of these preparations are erythromycin thiocynate premix and erythromycin phosphate powder (Ery-Mycin). Veterinary forms of erythromycin injectable (e.g., Erythro-100 and Gallimycin-100) are 100 mg/ml formulations intended for IM injection only; they should not be administered SC or IV. Doses of erythromycin are listed in Table 36.7. Erythromycin and other macrolide antibiotics are sometimes used as a penicillin alternative when penicillins have either failed or when there is allergy to penicillins. Infections treated by erythromycin include those caused by *Staphylococcus* spp., *Streptococcus* spp., *Arcanobacterium* spp., *Clostridium* spp., *Listeria* spp., *Bacillus* spp., *Erysipelothrix* spp., *Histophilus*, *Brucella* spp., *Fusobacterium* spp., *Pasteurella* spp., *Borrelia* spp., and *Mycoplasma* spp. Erythromycin has also been used as a treatment for undifferentiated bovine respiratory disease and for pig infections caused by *Erysipelothrix* and for pig respiratory infections caused by *Streptococcus* and *Pasteurella*. In poultry, erythromycin is used for treatment of respiratory infections caused by *Mycoplasma*. In foals, erythromycin is used, in combination with rifampin for treatment of pneumonia caused by *Rhodococcus equi*. However, there is some evidence that erythromycin administered alone may be equally efficacious. For this use in horses, azithromycin or clarithromycin have become more common (see Sections Azithromycin and Clarithromycin).

In small animals, erythromycin has been used to treat pyoderma caused by staphylococci (Noli and Boothe, 1999), respiratory infections caused by *Mycoplasma,* and diarrhea caused by *Campylobacter* organisms. However, because of pharmacokinetic studies in dogs and cats, inadequate oral absorption and need for frequent IV administration limits the practical use. When treating *Campylobacter,* erythromycin stopped the shedding but did not eliminate the organism. Respiratory infections have sometimes been treated with erythromycin, but other drugs (e.g., azithromycin) have become more common because of better spectrum, longer half-life, and fewer adverse gastrointestinal effects. Experience in cats has been very limited. Based on a pharmacokinetic study (Albarellos et al., 2011), the IV administration to cats had a very short half-life and effective concentrations sustained for only 1.5 hours. Intramuscular injections in cats produced pain at the injection site and would not be a practical route for repeated injections. Erythromycin in the ethylsuccinate form as tablets or oral suspension did not produce measureable serum concentrations (15 mg/kg) after oral administration to cats (Albarellos et al., 2011). In dogs the ethylsuccinate and estolate oral formulations were poorly absorbed (Albarellos et al., 2008). The half-life was also short and would require frequent administration. These finding raises doubt about the oral use of these formulations for treatment in dogs or cats.

Clinical Use of Erythromycin to Modify Gastrointestinal Motility

Although some nausea from oral erythromycin is possible, most of this effect is believed to be related to a drug-induced increase in gastrointestinal motility. This mechanism appears to be related to an increase in activation of motilin receptors, via release of endogenous motilin, or via cholinergic mechanisms in the upper gastrointestinal tract (Hall and Washabau, 1997; Lester et al., 1998). At small doses (1 mg/kg) erythromycin has been considered for use as a motility-stimulating drug in animals. In calves, administration of erythromycin, tylosin, or tilmicosin increased the rate of abomasal emptying, with erythromycin (8.8 mg/kg IM) producing the most significant effect (Nouri and Constable, 2007; Nouri et al., 2008; Wittek and Constable, 2005). In calves, these drugs increase the abomasal emptying rate and erythromycin (10 mg/kg IM) has been used to improve postoperative abomasal rate in dairy cows undergoing surgical correction of left abomasal displacement (Wittek et al., 2008). These properties of erythromycin are discussed in more detail in Chapter 46. Although 14-membered macrolides appear to have the most profound effect on the gastrointestinal tract (Table 36.4), there is also an effect from 16-membered macrolides such as tylosin and tilmicosin (Nouri and Constable, 2007).

Regulatory Considerations

Erythromycin has a 6-day withdrawal time when used according to label in cattle in the United States. Erythromycin added to feed or water for poultry has a withdrawal time of 1–2 days; the specific product label should be consulted for the exact withdrawal time. In the United States, erythromycin should not be administered to lactating dairy cattle because macrolides concentrate in the milk for a long time after treatment. However, Canadian labeling lists a milk withholding time of 72 hours after a dose of 2.2–4.4 mg/kg.

Tylosin

Pharmacokinetic data for tylosin are listed in Table 36.8. Tylosin has been used therapeutically to treat “pinkeye” (*Moraxella bovis*) in cattle; respiratory tract infections; swine dysentery; pleuropneumonia due to *Haemophilus parahemolyticus;* and other infections in cats, chickens (Ose and Tonkinson, 1985), quail (Jones et al., 1976), and turkeys (Wilson, 1984). Tylosin has been used more extensively as a feed additive in food-producing animals, such as swine, cattle, and chickens, among others (Wilson, 1984). Tylosin phosphate premix has been added to feed for cattle, pigs, or poultry, and tylosin tartrate (Tylan soluble) for the drinking water of poultry.

Residues from tylosin have been discussed in other papers (Knothe, 1977a, 1977b; Anderson et al., 1966). After administration to cattle there is a 21- and 14-day withdrawal time for slaughter for cattle and pigs, respectively. Tylosin concentrates in milk for a long time after administration and should not be administered to lactating dairy cattle. Specific product information should be consulted for withholding times when tylosin is administered in feed or water to pigs or poultry because withdrawal times can vary from 0 to 5 days, depending on the use.

Tylosin has also been used to treat diarrhea in dogs, which is discussed in Chapter 46 in more detail. This type of diarrhea has been characterized as "tylosin-responsive chronic diarrhea in dogs" (Westermarck et al., 2005). In these animals, tylosin has been effective at improving clinical signs that occur with or without organisms being identified.

Tilmicosin

Tilmicosin (Micotil) 300 mg/ml for SC injection (10–20 mg/kg) to cattle and sheep (10 mg/kg). Tilmicosin phosphate (Micotil 300) has been effective for treating bovine respiratory disease (Musser et al., 1996; Hoar et al., 1998; Jim et al., 1999). One study (Ose and Tonkinson, 1988) reports that 90% of the *Mannheimia haemolytica* and *Pasteurella multocida* isolates tested were susceptible to tilmicosin at concentrations of ≤6.25 μg/ml, and the drug was also active against *Mycoplasma,* including those from bovine isolates. Other organisms with in vitro susceptibility to tilmicosin include staphylococci and streptococci. Most gram-negative organisms other than those causing bovine respiratory disease are resistant.

Tilmicosin administered to calves with pneumonia were found to respond better when treated with 10 mg/kg SC tilmicosin than with a 20 mg/kg IM dose of oxytetracycline (Laven and Andrews, 1991). Like other macrolides, tilmicosin reaches high concentrations in lung tissues and this may account for efficacy treating bovine pneumonia (Gourlay et al. 1989). Resistance among cattle respiratory pathogens has been recognized (Musser et al., 1996), but treatment response in cattle with bovine respiratory disease was not associated with the MIC of the pathogens, and there was treatment success even when bacteria recovered had MIC values in the resistant range (McClary et al., 2011).

Tilmicosin also has been used as a prophylactic antibiotic (metaphylaxis) for administration to calves entering a feedlot situation. Tilmicosin reduced the incidence of pneumonia in susceptible calves when administered prophylactically as a single 10 mg/kg SC injection (Morck et al., 1993; Schumann et al., 1990). Tilmicosin used as a metaphylactic treatment in newly arrived feedlot calves reduced prevalence of bovine respiratory disease and improved growth of calves (Vogel et al., 1998).

The CLSI breakpoint (Table 36.5) for tilmicosin susceptibility is ≤8 μg/ml for cattle respiratory pathogens (*Mannheimia haemolytica*) and ≤16 μg/ml for swine respiratory disease pathogens. The currently approved dose is 10–20 mg/kg SC as a single treatment in cattle, and 10 mg/kg in sheep. After treatment with tilmicosin phosphate in cattle, there is a 28-day withdrawal time. Tilmicosin should not be administered to lactating dairy cattle because residues may persist in milk for more than 30 days.

Tilmicosin phosphate is approved for treatment of swine respiratory disease caused by *Actinobacillus pleuropneumoniae* and *Pasteurella multocida*. This form (Pulmotil) is administered as a feed additive and has been shown to be effective for controlling pneumonia in swine (Moore et al., 1996). There is a 7-day withdrawal time for slaughter when administered to swine.

Tilmicosin has also been used for treatment of pasteurellosis in rabbits (McKay et al., 1996). Single doses of 25 mg/kg SC were an effective treatment for pasteurellosis in rabbits.

Adverse Reactions to Tilmicosin

Injections of tilmicosin to horses, goats, swine, or nonhuman primates can be fatal. The heart is the target of toxicity in animals, perhaps mediated via depletion of cardiac intracellular calcium, resulting in a negative inotropic effect (Main et al., 1996). Epinephrine worsens the cardiac toxicity in pigs, but dobutamine has alleviated the cardiac depression in dogs (Main et al., 1996). The effects of toxicity are increased heart rate, arrhythmia, and depressed contractility. Injected doses of 20 and 30 mg/kg to pigs caused death, but oral tilmicosin in pigs produces no toxic effects. In cattle, injected SC doses of 50 mg/kg caused myocardial toxicity; 150 mg/kg was lethal. Doses as low as 10 mg/kg administered by the IV route have caused cardiac toxicity as well (Ziv et al., 1995).

The risk of cardiac toxicity is particularly important for humans. There are warnings on the tilmicosin label that accidental injection into humans has caused death. Published reports (Veenhuizen et al., 2006) indicate that several people have died as a result of tilmicosin administration.

Tulathromycin

The injectable formulation of tulathromycin (Draxxin) is 100 mg/ml for use as a single SC injection at 2.5 mg/kg. Tulathromycin is an azalide derivative of erythromycin, with three charged nitrogen groups; therefore it has been called a *triamilide* (Evans, 2005). These charged groups may be important to increase the intracellular concentrations compared to other macrolides. It is approved for use in cattle and pigs and has occasionally been used in other species. In cattle and pigs it is used for treating respiratory infections (bovine respiratory disease and swine respiratory disease), for which the pathogens have been discussed earlier in this chapter and CLSI breakpoints are listed in Table 36.5. In addition to these pathogens the label includes *Mycoplasma bovis* in this drug's indications. It is administered once (e.g., 2.5 mg/kg SC in cattle and IM in swine) and produces sustained drug concentrations in lung tissue for several days. It also is used to prevent bovine respiratory disease when administered to cattle (metaphylaxis) that are at risk for developing respiratory disease (Booker et al., 2007). Withdrawal times are 18 days for cattle and 5 days for swine. It has also been administered to other species and a dose of 2.5 mg/kg as a single injection has been used in other domestic food animals and zoo hoof stock.

There is limited evidence that tulathromycin may be useful for treating pulmonary infections in foals (Venner et al., 2007; Rutenberg et al., 2017). At 2.5 mg/kg IM once per week, it resolved pulmonary lesions and, except for diarrhea in some foals, was well tolerated. In a study of 240 foals endemic for infections caused by *Rhodococcus equi,* treated with 2.5 mg/kg IM, once per week, it was effective, but not as effective as the combination of azithromycin–rifampin (Rutenberg et al., 2017). However, it is less active than other agents against *Rhodococcus equi* and is not recommended (Giguère et al., 2011).

Clarithromycin

Clarithromycin (Biaxin®) is semisynthetically derived from erythromycin. It is primarily used in people because it is tolerated better than erythromycin, has a broader spectrum, and concentrates in leukocytes. Clarithromycin in combination with ranitidine and bismuth (Tritec®) is also used to treat *Helicobacter pylori* infections in people. In dogs, clarithromycin does not have pharmacokinetic features that are as favorable as those of azithromycin (the half-life is not as long) and the use is rare.

Most veterinary experience has been in foals, where clarithromycin has been investigated as a potential treatment for respiratory infections. It has more activity against *Rhodococcus equi* isolated from foals than other macrolides (Jacks et al., 2003; Riesenberg et al., 2014; Berghaus et al., 2013). In foals, clarithromycin is absorbed orally and has a half-life of 4–6 hours, depending on the study (Table 36.6). Not shown in Table 36.6 is the effect of coadministration of rifampin on clarithromycin concentrations in foals. Because of induction of enzymes and transporters, coadministration with rifampin decreases plasma drug concentrations by over 90%, which is discussed in more detail in Section Rifampin (Rifampicin).

The concentrations in the respiratory ELF, bronchial/alveolar epithelial cells, and BAL cells of foals is many fold higher than plasma drug concentrations – reaching levels that are over 30–40 times higher in the ELF and over 300–1800 times higher in BAL cells (Peters et al., 2011, 2012). However, the concentrations do not persist in tissues for as long as azithromycin (Suarez-Mier et al., 2007). Oral absorption in foals was 57% (Womble et al., 2006) and 41.5% (Berlin et al., 2016), compared to 70–75% in dogs. In foals, oral clarithromycin at a dose of 7.5 mg/kg every 12 hours produces concentrations sufficient for treatment of *Rhodococcus equi* infections (Jacks et al., 2002; Giguère et al., 2011). It has been more successful at this dose than azithromycin (Giguère et al., 2004). It is also metabolized in horses to 14-hydroxyclarithromycin, which is microbiologically active and contributes to the activity (Peters et al., 2011, 2012; Berlin et al., 2016).

Gamithromycin

Gamithromycin (Zactran) is a 15-membered ring (like azithromycin and tulathromycin). The mechanism of action is the same as other macrolides. Gamthromycin has a spectrum of activity that is limited to gram-positive bacteria and some gram-negative bacteria that cause respiratory diseases in cattle (e.g., *Mannheimia haemolytica, Mycoplasma,* and *Pasteurella multocida*). Susceptibility information and pharmacokinetics are listed in Tables 36.5 and 36.6. Like other long-acting macrolides, the half-life is long (Giguère et al., 2011, Table 36.6) with long persistence in lungs, which prolongs the drug concentration at the site of infection.

In cattle, several studies have established the efficacy of gamithromycin for treatment of bovine respiratory disease caused by *Mannheimia haemolytica, Pasteurella multocida*, *Histophilus somni*, and *Mycoplasma bovis* (Torres et al., 2013a, 2013b; Lechtenberg et al., 2011a, 2011b, 2011c, 2011d). In two studies it had a higher morbidity rate and retreatment rate than cattle treated with tulathromycin (Torres et al., 2013a, 2013b), but was otherwise equivalent. It is also effective for treating infections caused by *Mycoplasma bovis*. It also may be used for control of respiratory disease in beef and nonlactating dairy cattle at high risk of developing bovine respiratory disease (metaphylaxis) associated with *Mannheimia haemolytica* and *Pasteurella multocida*.

The MIC values for *Rhodococcus equi* are low and a IM dose of 6 mg/kg has been investigated for treatment of horses (Berghaus et al., 2012; Hildebrand et al., 2015). Even though it was effective in foals with bronchopneumonia, it had a higher incidence of adverse effects in foals that included colic and hind limb lameness. Almost 60% of treated foals showed reactions to the administration of gamithromycin.

Tildipirosin

Tildipirosin (Zuprevo) is a 16-membered ring (like tilmicosin) macrolide antimicrobial with three charged nitrogen atoms (like tulathromycin), which is currently limited to the treatment and control/prevention of bovine respiratory disease and the treatment of swine respiratory disease, although it has also been used for the control/prevention of swine respiratory disease. The mechanism of action is the same as for other macrolides. Pharmacokinetics and other properties were reported by Menge et al. (2012) and Rose et al. (2013). Tildipirosin has a spectrum of activity that is limited to gram-positive bacteria and some gram-negative bacteria that cause respiratory diseases in cattle and pigs (e.g., *Mannheimia haemolytica, Mycoplasma, Pasteurella multocida, Actinobacillus pleuropneumoniae, Bordetella bronchiseptica,* and *Haemophilus parasuis*). *Escherichia coli* and *Pseudomonas aeruginosa* are resistant. Some *Staphylococcus* spp. and *Streptococcus* spp. may be susceptible. There is evidence for bactericidal activity against *Mannheimia haemolytica*, bovine *Pasteurella multocida, Histophilus somni, Haemophilus parasuis,* and *Actinobacillus pleuropneumoniae*, but bacteriostatic activity against *Bordetella bronchiseptica*.

Pharmacokinetics in cattle (Table 36.6) shows that the half-life is long and bioavailability from injection in cattle is 79%. The volume of distribution is larger than other macrolide antibiotics, with a volume of distribution in cattle of 49 l/kg. The lung concentrations in cattle are over 150 times the plasma drug concentrations, with a half-life of 10 days. Bronchial fluid concentrations are approximately 40 times the plasma drug concentrations, with a half-life of 11 days.

In pigs, the plasma half-life is 106 hours (4.4 days), with a peak concentration of 0.9 μg/ml after IM injection of 4 mg/kg. The lung concentrations in pigs were approximately 80 times higher than plasma concentrations, with a half-life of 6.8 days. The bronchial fluid concentrations were 680 times higher than plasma drug concentrations at 5 days after injection. Tildipirosin, like other macrolides, exerts therapeutic benefits not solely explainable by antibacterial activity and may have immunomodulatory effects.

Tildipirosin has been approved for the treatment and control/prevention of bovine respiratory disease associated with *Mannheimia haemolytica, Pasteurella multocida,* and *Histophilus somni* and in some European countries for the treatment of swine respiratory disease associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Bordetella bronchiseptica,* and *Haemophilus parasuis*.

Azithromycin

Azithromycin (Zithromax®) is the first drug in the class of azalides approved for people, but it also is administered frequently to small animals, exotic species, and horses. Azithromycin has better oral absorption, is better tolerated, has a much longer half-life (especially in tissues), and has a broader spectrum of activity than erythromycin.

Azithromycin is active against gram-positive aerobic bacteria (staphylococci and streptococci) and anaerobes. However, the activity against staphylococci is not as good as erythromycin. It has some activity against gram-negative bacteria such as *Haemophilus* but limited activity against enteric gram-negative bacteria, and ineffective against *Pseudomonas aeruginosa*. It has activity against many intracellular organisms, including *Chlamydophilia* (formerly called *Chlamydia*) and *Toxoplasma.* It is also active against mycobacteria and *Mycoplasma* (Lode et al., 1996).

The primary pharmacokinetic difference between azithromycin and erythromycin is the long half-life and high concentration in tissues. Pharmacokinetic properties are shown in Table 36.6. Azithromycin reaches high concentrations in tissues, particularly leukocytes, macrophages, and fibroblasts. The tissue concentration can be as much as 100 times serum concentrations and concentrations in leukocytes can be at least 200–300 times the concentrations in serum (Panteix et al., 1993). In cats, the serum half-life is 35 hours, tissue half-lives vary from 13 to 72 hours, and the volume of distribution is 23 l/kg (Hunter et al., 1995). In dogs, it also exhibits rapid uptake and persistent concentrations in tissues; the volume of distribution is 12 l/kg, and plasma and tissue half-lives are 29 and 90 hours, respectively (Shepard and Falkner, 1990). Oral absorption is high, with bioavailability values of 58% in cats (Hunter et al., 1995) and 97% in dogs (Shepard and Falkner, 1990). In people, azithromycin is absorbed much better on an empty stomach (Lode et al., 1996) but the effect of feeding on oral absorption has not been explored in cats or dogs.

There also is interest in administering azithromycin to horses (Davis et al., 2002; Jacks et al., 2002, 2003; Suarez-Mier et al., 2007). Davis et al. (2002) showed that oral absorption was 39% and had a plasma half-life of 18 hours in foals. More importantly, the drug persisted in leukocytes and alveolar macrophages for at least 120 hours after a single dose at concentrations greater than 5.0 μg/ml, with a half-life in leukocytes of over 49 hours. As in other species, concentrations in PMNs were over 200 times the plasma concentrations. It had a volume of distribution in horses of 12 l/kg, which probably accounts for the long persistence in inflammatory cells.

Like other long-acting macrolide antibiotics, azithromycin produce high concentrations in tissues and leukocytes, even after the plasma concentrations have declined below detectable levels (Girard et al., 1990). Intracellular stores of azithromycin in leukocytes also can serve as a mode of delivery of azithromycin to infected tissues, especially early abscesses, since the leukocytes are attracted to these sites via chemotaxis (Girard et al., 1993). The immunomodulatory effects of azithromycin have been studied extensively and discussed previously in this chapter (Parnham et al., 2014). Beneficial effects of azithromycin are attributed, in part, to these effects on inflammatory cells and immune function.

Clinical Use of Azithromycin

Azithromycin has become popular for treating infections in dogs, cats, exotic animals, and birds. Results of treatment of intracellular infections caused by *Toxoplasma* spp. and *Mycobacterium* spp. have been conflicting in people and are not yet reported for animals. Because of the long half-life and persistence of drug in tissues, the regimen employed in people is to administer a dose once daily for 3–5 days. Thereafter, effective drug concentrations are expected in tissues for up to 10 days. In dogs, doses of 5–10 mg/kg once daily orally for 1–5 days have been suggested. In cats, doses of 5–10 mg/kg once daily or every other day or one dose two to three times a week orally have been used.

Despite the popularity of azithromycin for treatment of infections in dogs and cats, there is little clinical evidence published to demonstrate benefits over other drugs. In shelter cats with upper respiratory infections, it was no better than amoxicillin for treatment (Ruch-Gallie et al., 2008). In cats with chlamydophilosis (*Chlamydophila felis*) it was ineffective for clearing the infection. In dogs it has been effective for some skin infections based on limited reports, but was not as effective as other agents for treatment of Rocky Mountain spotted fever (*Rickettsia rickettsii*) (Breitschwerdt et al., 1999).

There are several reports of azithromycin clinical use in foals with pulmonary infections, such as those caused by *Rhodococcus equi.* Because of favorable pharmacokinetics, cited above, plasma, leukocyte, and alveolar macrophage concentrations persist long enough to allow for every-other-day administration. Based on this work the dose for foals is 10 mg/kg every 24 hours initially, followed by treatment every 48 hours orally. When azithromycin was administered orally to foals (10 mg/kg every 48 hours) it effectively reduced the pneumonia attributed to *Rhodococcus equi* (Chaffin et al., 2008). The use of azithromycin and other macrolides for treatment of *Rhodococcus equi* infections in foals was summarized in an ACVIM Consensus Statement (Giguère et al., 2011).

Safety of Azithromycin

Azithromycin is generally well tolerated. In people, gastrointestinal disturbances are the most common side effects (nausea, vomiting, diarrhea, abdominal pain). In dogs, high doses may cause vomiting. From the clinical reports, it appears to have been well tolerated in foals but transient diarrhea is possible. Adult horses may be more prone to developing diarrhea and more caution is urged with clinical use in these animals.

Erythromycin is well known to decrease the activity of drug-metabolizing enzymes in the liver. This can increase the toxicity of some drugs administered concurrently. Although azithromycin is reported to have less effect on the hepatic enzymes, some caution is needed when combining azithromycin with other drugs.

Lincosamide Antibiotics

Lincosamides are a group of monoglycoside antibiotics containing an amino acid-like side chain. There are two antibiotics within this group: lincomycin and clindamycin. Lincomycin and clindamycin are structurally similar. Lincomycin has a hydroxyl moiety at the 7 position of the molecule, and clindamycin contains a chlorine at this position (Figure 36.4), making

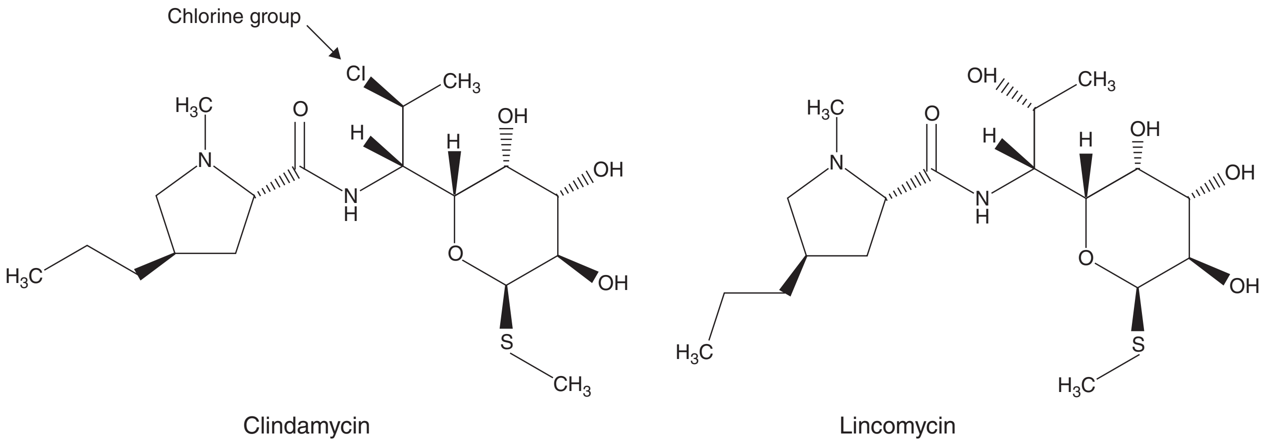


Figure 36.4 The chemical structures of clindamycin (left) and lincomycin (right). Structural difference is in the chlorine group on clindamycin.

clindamycin a more active molecule against bacteria than its parent molecule, lincomycin, and better absorbed orally. The lincosamides, like the macrolides, are used primarily to treat gram-positive infections in cases where there is resistance or intolerance to penicillins. Clindamycin also is a common drug for treatment of anaerobic infections. Common infections treated with lincosamides include infections involving *Staphylococcus* spp. and *Streptococcus* spp. (Burrows, 1980).

Lincomycin

Source and Chemistry

Lincomycin is the antibiotic produced by *Streptococcus lincolnensis* var. *lincolnensis,* discovered in the 1950s; its name comes from cultures of soil that originated in Lincoln, Nebraska. Veterinary formulations were first developed in the 1960s. Lincomycin is a weak base with a pK_a of 7.6 (Riviere et al., 1991).

Formulations

Lincomycin is available as an oral premix for pigs and chickens (Lincomix) and a soluble powder for drinking water (Lincomix). Lincomycin hydrochloride oral syrup and tablets have been used for dogs and cats (Lincocin), as well as lincomycin hydrochloride injection, but use in small animals is not as common as it once was. Ruminants and horses should not be exposed to lincomycin-supplemented feed. The toxicity is described in Section Adverse Effects and Precautions. There has been combination products of lincomycin and spectinomycin available in the past (for example Linco-Spectam), but this product has been discontinued in many countries. Veterinarians should consult local availability in each country of use.

Mechanism of Action and Spectrum

Lincomycin inhibits protein synthesis in the microbial cell by binding to the 50S ribosomal subunit in much the same way described for macrolides. Other antibiotics, such as erythromycin and clindamycin, function similarly by binding at different sites to the same ribosomal subunit. The spectrum of activity is similar for macrolides and lincosamides, with exceptions listed for individual drugs in this chapter. Bacteria with resistance to macrolides usually show cross-resistance to lincosamides. Macrolides and lincosamides should not be used together because this may produce a decrease in the overall efficacy against the microbe due to one bound antibiotic physically overlapping the binding site of another (Burrows, 1980).

Absorption and Distribution

Lincomycin is rapidly but incompletely absorbed when administered orally to animals, with one report stating that lincomycin oral absorption in swine given 10 mg/kg is in the range 20–50% (Hornish et al., 1987). Peak serum levels in most animals are reached within 60 minutes after an oral dose and within 2–4 hours after IM injection. Lincomycin is well distributed in the body, with highest tissue concentrations in the liver and kidneys, while very low levels are obtained in the CSF (Burrows, 1980; Ford and Aronson, 1985; Kleckner, 1984). The Vd in animals ranges from 1 to 1.3 l/kg.

Metabolism and Excretion

The half-life after oral, IM, or IV administration is approximately 2–4 hours. Most of the oral dose, measured as ^{14}C-labeled lincomycin, was recovered in the feces and 14% in the urine after a single oral administration to the dog (Kleckner, 1984); thus, biliary secretion of lincomycin appears to be an important route of elimination. After a single IM injection, 38% of the dose was found in the feces and 49% in the urine of the dog. Urine excretion of the radiolabeled drug was complete in 24 hours and fecal excretion was complete within 48 hours for both dosing routes. It is not known whether this radioactivity was associated with an unchanged/unmetabolized lincomycin or with the metabolites of this compound. An unpublished report cited by Hornish et al. (1987) stated the parent drug was the primary form present in the urine of dogs and humans.

Because of the potential for residues in meat, the metabolism and excretion of lincomycin have been studied more extensively in swine and chickens (Hornish et al., 1987). Lincomycin concentrations are highest in the liver and kidney, with low, albeit detectable, levels in muscle and skin. Lincomycin can pass unchanged from the body via the bile and feces or urine or can be metabolized to the glucuronide, *N*-demethyl lincomycin, or lincomycin sulfoxide forms by the liver. Swine given oral doses of lincomycin showed that 11–21% was excreted into the urine: 50% unchanged lincomycin, trace amounts of *N*-demethyl lincomycin, no lincomycin sulfoxide or glucuronide forms, and the rest labeled “unidentified substances.” The feces contained the remainder of the excreted lincomycin: 17% unchanged lincomycin, possible trace amounts of lincomycin sulfoxide, and 83% uncharacterized metabolites (Hornish et al., 1987). Similarly conducted studies in chickens treated orally for 7 days with lincomycin showed that the excreta contained ≈80% lincomycin, ≤10% lincomycin sulfoxide, ≤5% *N*-demethyl lincomycin.

Adverse Effects and Precautions

Dogs and cats have few adverse reactions to lincomycin. Loose stools in the dog and vomiting in the cat have been the major side effects reported (Kleckner, 1984). Pigs may occasionally develop diarrhea and/or swelling of the anus within the first 2 days of treatment and will self-correct within a week after withdrawal from the antibiotic.

The most serious adverse effect from lincomycin reported in people is that of pseudomembranous colitis. This is a serious disease in people caused by an overgrowth and production of toxin from *Clostridium difficile,* which may be fatal. In animals with fermenting gastrointestinal tracts (horses, ruminants, rabbits, hamsters, chinchillas, and guinea pigs) there also is a high risk of gastrointestinal bacterial overgrowth with *Clostridium* spp. from lincomycin treatment. Severe enteritis, enterocolitis, may lead to diarrhea and death. Other bacteria also have been implicated in this reaction, such as *Salmonella* spp. or *E. coli* (Burrows, 1980; Plenderleith, 1988). Lincomycin-induced enterocolitis has been reported for rabbits (Maiers and Mason, 1984; Thilsted et al., 1981; Rehg and Pakes, 1982), horses (Raisbeck et al., 1981; Plenderleith, 1988), sheep (Bulgin, 1988), and large ruminants (Plenderleith, 1988). Lincomycin has been reported to produce ketosis in dairy cows (Rice and McMurray, 1983).

Clinical Use

There are 85 products listed on the FDA list of approved drugs for animals. These products are in oral and injectable form for pigs, dogs, cats, and poultry. Lincomycin is used to treat gram-positive aerobic and anaerobic infections in patients for many of the same indications for which one would use erythromycin or other macrolides. In dogs and cats, lincomycin has been used to treat penicillin-resistant or suspected penicillin-resistant strains of *Staphylococcus* spp. and *Streptococcus* spp. bacteria found in bone, the upper respiratory tract, and the skin. Although it has been used for skin infections, it is not as popular as it once was (Noli and Boothe, 1999). Oral doses in dogs and cats generally are 22 mg/kg every 12 hours orally. The use of lincomycin to treat bacterial infections in dogs and cats has been largely replaced by clindamycin therapy (see Section Clindamycin).

Lincomycin has been utilized to treat bacterial arthritis in swine caused by *Staphylococcus* spp., *Streptococcus* spp., *Erysipelothrix* spp., and *Mycoplasma* spp., and pneumonia caused by *Mycoplasma* spp. Lincomycin has been added to the feed and water to control swine dysentery and *Mycoplasma* infections (Rainier et al., 1980; Hamdy, 1978; Hamdy and Kratzer, 1981). Injections have been administered to pigs for *Mycoplasma* infections (11 mg/kg every 24 hours IM). In cattle and sheep lincomycin has been injected IM for treatment of septic arthritis and mastitis and to control *Mycoplasma* infections. It should never be administered orally to these animals because of risk of inducing enteritis.

In broiler chickens, lincomycin has been used as a feed additive to increase the rate of weight gain and improve feed efficiency (this use has been phased out in the United States), in addition to treating necrotic enteritis in this species. The addition of 2 g/ton of lincomycin to the feed of broilers resulted in a significant decrease in the incidence of necrotic enteritis (Maxey and Page, 1977). Lincomycin has also been used with success in psittacines (Mandel, 1977). Lincomycin use in the eyes of rabbits has also been reported (Kleinberg et al., 1979). Topical corneal administration of 1% lincomycin in water to rabbits showed local therapeutic levels could be maintained from 30–45 minutes to 2 hours postdose in the cornea, aqueous humor, and iris-ciliary body and that deepithelialization of the corneal epithelium served to enhance the ocular topical absorption of this antibiotic.

Sheep, goats, and calves have been treated with parenteral lincomycin–spectinomycin antibiotic combinations for gram-positive and gram-negative respiratory tract infections. The lincomycin–spectinomycin combination (Linco-Spectam, 50 mg lincomycin with 100 mg spectinomycin per ml) at a dose of 1 ml/10 kg body weight IM has been used to treat foot rot in sheep caused by *Bacteroides nodosus* with better success than systemic penicillin–streptomycin therapy (Venning et al., 1990). However, in many countries the combination product of lincomycin–spectinomycin has been discontinued (see the discussion in section Spectinomycin).

Regulatory Considerations

When added to feed for poultry and pigs, the slaughter withdrawal time ranges from 0 to 6 days, depending on the preparation and dose. When injected in pigs, the withdrawal time for slaughter is 2 days. Because a large number of products are listed on the FDA approved drug list, consult the package insert for specific recommendations.

Clindamycin

Source and Chemistry

Clindamycin chemically is 7-chlorolincomycin, a derivative of lincomycin and an antibiotic produced by *Streptococcus lincolnensis* var. *lincolnensis*. The replacement of the hydroxyl group at the C7 position of the lincomycin molecule by a chloride results in a more active antibacterial effect when compared to lincomycin. The chemical structure of clindamycin is shown in Figure 36.4. It is a weak base with a pK_a of 7.6. Both clindamycin hydrochloride (HCl) and clindamycin palmitate are for oral administration. Clindamycin HCl is directly active when administered, whereas the palmitate form must be converted to clindamycin in the small intestine. Clindamycin palmitate is more palatable than clindamycin HCl. Clindamycin phosphate is the parenteral form of clindamycin and must undergo hydrolysis in the plasma for it to become active.

Mechanism of Action and Resistance

Clindamycin exerts its antibiotic activity by inhibiting protein synthesis at the 50S ribosomal subunit (Hedstrom, 1984) in a manner identical to that described for lincomycin. Resistance to clindamycin is most often caused by methylation of the 23S rRNA, which is the same mechanism that is most common for macrolide antibiotics. This mechanism of resistance is mediated by *erm* genes. Bacteria carrying these genes can be resistant to both macrolides and clindamycin. The other mechanism of resistance for macrolides is the efflux pump mediated by the *mef* gene. Clindamycin is not affected by this gene. Although modification of the 23S ribosome is the most common mechanism of resistance, if bacteria are resistant to macrolides because of the efflux pump mechanism, they may still be susceptible to clindamycin. The third mechanism of resistance, enzymes directed at the drug, is uncommon. Bacteria resistant to lincomycin are also resistant to clindamycin.

Spectrum of Activity

The chlorine substitution (Figure 36.4) produces higher activity against some bacteria than lincomycin. Clindamycin has been reported to be as much as 20 times more potent than lincomycin in the treatment of *Staphylococcus* and *Streptococcus* infections in humans (Harvey, 1985). Clindamycin is active against aerobic species of organisms, including *Staphylococcus, Streptococcus, Actinomyces, Nocardia, Mycoplasma,* and *Toxoplasma*. Because macrolide efflux is the predominant mechanism of macrolide resistance for *Streptococcus* spp., clindamycin remains active against most streptococci because they are not affected by this mechanism. The anaerobic bacterial spectrum of activity includes *Bacteroides fragilis, Fusobacterium* spp., *Peptostreptococcus* spp., and *Clostridium perfringens* (Harari and Lincoln, 1989). Clindamycin is not active against aerobic and facultatively anaerobic gram-negative bacilli such as the Enterobacteriaceae or *Pseudomonas* spp. *Pasteurealla* spp. (gram-negative aerobe) isolated from bite wounds of small animals are usually resistant to clindamycin.

Although most staphylococci are susceptible to clindamycin, approximately 25–36% of *Staphylococcus* spp. may be resistant to clindamycin, depending on the study and region from which the bacteria were isolated. Most methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) isolated from dogs are resistant to clindamycin. On the other hand, many community-acquired methicillin-resistant *Staphylococcus aureus* may be susceptible to clindamycin.

In small animals, anaerobic infections are one of the major uses of clindamycin. One report (Jang et al., 1997) indicated that 83% of *Bacteroides* from small animals were susceptible to clindamycin and 80% of the *Clostridium.* Clindamycin resistance among *Bacteroides* is mediated by the *erm* gene and increased rates of resistance among anaerobes may reflect the prevalence of this gene. Most *Fusobacterium* spp. are also susceptible to clindamycin. An additional organism for which there is activity is *Toxoplasma,* but the clinical use of clindamycin for treating toxoplasmosis in cats is controversial (described in more detail in Section Clinical Use).

The CLSI breakpoint for susceptibility testing is ≤0.5 μg/ml, 1–2 μg/ml, and ≥4 μg/ml for susceptible, intermediate, and resistant categories, respectively (CLSI, 2015). Clindamycin may be used to test for lincomycin susceptibility, although clindamycin may be more active against some staphylococci than lincomycin.

Pharmacokinetics – Pharmacodynamics

Clindamycin exerts a bacteriostatic rather than a bactericidal effect on bacteria; therefore, it is important to maintain the plasma drug concentration above the MIC throughout the dose interval. The area-under-the-curve (AUC) to MIC ratio (AUC/MIC) for the free drug concentration is the best predictive parameter for clindamycin efficacy. The AUC/MIC target for a bacteriostatic effect is approximately 25. Protein binding is 92–95% in dogs and 91.5–94.5% in cats (protein binding tends to be lower at high concentrations ranging from 0.5 μg/ml to 5 μg/ml). Therefore, PK/PD calculations should use the free drug fraction (fraction unbound).

Absorption and distribution: Clindamycin is better absorbed from the gastrointestinal tract than lincomycin, yielding higher plasma concentrations (Nichols and Keys, 1984). Unlike lincomycin, the presence of food does not appear to affect oral absorption of clindamycin. Oral absorption was 73% in dogs after capsule administration and highly absorbed from IM injection (87%). When injected SC, the absorption is slow, producing a “flip-flop” effect and a longer half-life. Other pharmacokinetics are shown in Table 36.9. At doses administered to cats (Brown et al., 1989, 1990) 5.5 and 11.0 mg/kg oral doses maintained a serum MIC above that necessary for most *Staphylococcus aureus* infections and that the 11.0 and 22.0 mg/kg doses gave serum concentrations above the MIC for many susceptible anaerobes. Cats may be reluctant to accept the oral liquid form of clindamycin because of poor palatability.

In one study, it was reported that clindamycin is too painful for IM administration (Budsberg et al., 1992), but in another study, IM administration of a buffered, more concentrated 20% solution was better tolerated. SC administration may be better tolerated than IM injections (Lavy et al., 1999).

Little is known in most exotic or zoo species, but in sea turtles clindamycin had extremely rapid clearance and a short half-life. There was very little oral absorption.

Table 36.9 Pharmacokinetics of clindamycin in animals

Species	Dose (mg/kg)	Half-life (hour)	Volume of distribution (Vd) (l/kg)[b]	AUC (μg h/ml)	Peak Concentration (C_{max}) (μg/ml)	Reference
Cats	11–33 oral	9.02	3.75	31-42 at 11 mg/kg	6.6-7.4 at 11 mg/kg	Boothe et al., 1996; Brown et al., 1989[a]
	5.5 oral	4.25	–	6.7	1.9	Saridomichelakis et al., 2011
	11 oral	9.92	–	18.35	3.3	Saridomichelakis et al., 2011
	11 IV	3.24	1.5	34.99	–	Budsberg et al., 1992
	11 IM	3.91	–	35.7	5.3	Budsberg et al., 1992
	10 IV	2.1	1.23	24.28	–	Lavy et al., 1999
	10 IM	7.1	–	30.1	4.4	Lavy et al., 1999
Dogs	10 SC	5.2	–	87.63	20.8	Lavy et al., 1999
	11 IV	4.37	3.08	22.5	–	Batzias et al., 2005
	11 oral	4.37	2.84	16.2	3.25	Batzias et al., 2005

[a] Data represents a mean of the published values.
[b] Values for Vd represent Vd/F for oral doses.

These results in sea turtles suggest that it would be impractical to use in these animals (Harms et al., 2011).

In dogs and cats the volume of distribution is over 1 l/kg and there is good penetration into respiratory secretions, pleural fluid, the prostate, bones, and joints, but with low concentrations in the CSF. The concentrations of clindamycin in phagocytes are 10- to 20-fold (and as high as 40) times the plasma concentrations (Harari and Lincoln, 1989). Despite the high intracellular concentrations of clindamycin in phagocytes, intracellular killing is poor (Yancy et al., 1991), perhaps because the drug is sequestered in subcellular sites. Macrophages take up clindamycin by an active transport mechanism and concentrate clindamycin up to 50 times the extracellular concentration (Dhawan and Thadepalli, 1982). Because phagocytes are the cells most likely to enter infected tissues, such as abscesses, it is possible for clindamycin to be transported to an abscess to produce high concentrations in these sites (Yancy et al., 1991). Clindamycin also crosses the placental barrier, but its safety during pregnancy has not been determined for animals.

Metabolism and Excretion

Clindamycin HCl requires no metabolism to be active once administered orally. Clindamycin phosphate requires hydrolysis to occur in the plasma to be active; similarly, clindamycin palmitate requires the removal of the palmitate moiety in the small intestine to be active. The commercial form for small animals (Antirobe) is clindamycin HCl; other formulations are available for people. Elimination half-lives are shown in Table 36.9. For dogs and cats the half-life varies among studies, dose, and formulation. Generally, the half-life is long enough after administration of the oral formulation that once- or twice-daily administration is sufficient.

Clindamycin metabolites are much like those described for lincomycin. In dogs, 36% of the administered dose of clindamycin is excreted unchanged by the bile and urine. The balance of the dose appears to be active or inactive metabolites, 28% excreted by the liver in the glucuronide form (no antimicrobial activity), 28% as clindamycin sulfoxide (25% of the antimicrobial activity of the parent antibiotic), and 9% as *N*-demethyl clindamycin, which has four to eight times the antimicrobial activity of the parent compound (Dhawan and Thadepalli, 1982). The bile is the major excretion route. The presence in the colon of people administered clindamycin suppressed microbial activity for as long as 2 weeks after the discontinuation of therapy.

Adverse Effects and Precautions

Like lincomycin, the most serious adverse effect in humans is pseudomembranous colitis, from overgrowth of *Clostridium difficile*. This has not been a reported problem in animals. In dogs and cats, vomiting and diarrhea are possible but they are transient and not serious. However, gastrointestinal problems such as those discussed for lincomycin in ruminants, horses, rabbits, and rodents, are possible, and the same precautions apply that were discussed for lincomycin. Although pseudomembranous colitis from *Clostridium difficile* has not been described in animals, bacterial overgrowth and diarrhea are still possible with oral administration of clindamycin to dogs and cats.

Greene et al. (1992) reported that administration of 25 and 50 mg/kg clindamycin HCl to cats, divided in two doses, produced diarrhea and vomiting as the most common clinical signs associated with oral therapy. The highest frequency for both of these clinical signs occurred in the 50 mg/kg treatment group and was thought to be related to either a direct irritant effect on the gastrointestinal tract or some effect on intestinal water absorption. As reported for other oral drugs in cats (for example, doxycycline hyclate) oral administration of clincamycin hydrochloride has been associated with esophageal injury (Beatty et al., 2006).

A study was performed in cats to ascertain the effect of prolonged clindamycin therapy on vitamin K-dependent blood clotting times (Jacobs et al., 1989). The study showed that factor VII levels did not significantly change in cats treated with a total daily dose of 25 mg/kg orally once daily for 6 weeks compared to controls.

Clinical Use

Clindamycin possesses an antimicrobial spectrum similar to that of lincomycin, but it is much more extensively used clinically than lincomycin because of higher activity against anaerobes, increased potency, and more complete oral absorption. Clindamycin has been used to treat wounds, abscesses, osteomyelitis, and periodontal diseases caused by susceptible organisms in dogs and cats. Clindamycin is found in high concentrations in the prostate, making it an acceptable choice for treating bacterial prostatitis when caused by gram-positive organisms.

The use of clindamycin for treating toxoplasmosis is controversial. Lappin et al. (1989) performed a retrospective study of cats diagnosed with *Toxoplasma gondii* infections and found that those cats treated with clindamycin resolved all clinical signs of the disease except those lesions involving the eyes. Clindamycin alone or in combination with a corticosteroid helped to resolve the active retinochoroiditis and the anterior uveitis associated with this disease. Even though clindamycin may help clinical signs associated with toxoplasmosis, it may not help to clear organisms from the CNS or the eye. In experimentally infected cats, there was a paradoxical effect in that cats with toxoplasmosis treated with clindamycin had a worsening of clinical signs. As discussed in more detail by Davidson et al. (1996), this paradoxical effect may be due to an inhibition of intracellular killing of organisms by clindamycin.

Clindamycin has been effective in dogs with experimentally induced posttraumatic osteomyelitis caused by *Staphylococcus* spp. (Braden et al., 1987, 1988). An oral dose of 11 mg/kg twice daily for 28 days was found to be efficacious in treatment of these infected dogs, resulting in a 94% recovery rate in the clindamycin-treated dogs. Clindamycin also has been shown to be effective for treatment of superficial and deep pyoderma in dogs and is a common choice as an alternative to β-lactam antibiotics (Harvey et al., 1993; Noli and Boothe, 1999; Scott et al., 1998). Although 11 mg/kg every 24 hours has been used to treat staphylococcal infections (pyoderma) in dogs, dosing of 11 mg/kg every 12 hours is used by many veterinarians for treating most *Staphylococcus* spp. infections. Once per day at 11 mg/kg may be sufficient for bacteria with MIC values ≤ 0.5 μg/ml; twice-daily administration should be used if the bacteria have MIC values in the intermediate range of 1–2 μg/ml.

Miscellaneous Antibiotics

Bacitracin

Bacitracin is a complex labile polypeptide consisting of five to ten separate chemical components first isolated from a *Bacillus subtillus* contaminated wound in 1943 (Teske, 1984). Bacitracin A ($C_{66}H_{103}N_{17}O_{16}S$) is the major component of this mixture and accounts for most of the antibiotic activities. Bacitracin inhibits peptidoglycan synthesis in bacteria by nonspecifically blocking phosphorylase reactions, some of which occur during cell wall synthesis (Lancini and Parenti, 1982). Development of resistance to bacitracin is rare.

Bacitracin is not absorbed from the gastrointestinal tract when given orally. Systemic administration has resulted in a high incidence kidney injury (albuminuria, cylindruria, azotemia), in addition to pain, induration, and petechiae at the site of injection. In contrast, bacitracin is nonirritating and rarely induces allergic reactions when used topically. Bacitracin (bacitracin, bacitracin methylenedisalicylate, bacitracin manganese, zinc bacitracin) has been used as a feed additive in livestock, but its most common use today is in topical applications to treat susceptible skin, ear, and eye infections. Bacitracin inhibits many organisms found on skin, such as hemolytic and nonhemolytic *Streptococcus* spp., coagulase-positive *Staphylococcus* spp., and some *Clostridium* spp., and it is often combined with other antibiotics that have a gram-negative spectrum of activity (polymyxin B, neomycin). Zinc bacitracin administered topically may increase the activity of bacitracin due to zinc's astringent properties, which decrease inflammation (Harvey, 1985).

Novobiocin

Novobiocin is a dibasic acid ($pK_a = 4.3$ and 9.1) derived from coumarin and is utilized clinically as a mono- (Na^+) or dibasic- (Ca^{++}) salt form. Novobiocin possesses activity against both gram-positive and gram-negative bacteria but is more active against the gram–positive bacteria, in particular *Staphylococcus* species. Other susceptible organisms include *Neisseria* spp., *Haemophilus* spp., *Brucella* spp., and some strains of *Proteus* spp. It may be used as an alternative to penicillins in cases involving penicillin-resistant *Staphylococcus* spp., although other penicillin substitutes (cephalosporins, macrolides, clindamycin) are better clinical choices.

Novobiocin has several toxic effects on bacteria, but its exact mechanism and site of action are unknown. Novobiocin has been shown to cause nonspecific inhibition of cell wall synthesis by inhibiting formation of alternating *N*-acetylmuramic acid pentapeptide and *N*-acetylglucosamine residues; it also inhibits teichuronic acid in some species of bacteria. The concentrations needed to inhibit these cell wall components are greater than the minimal concentration needed to inhibit growth, suggesting these effects on bacteria are secondary effects. DNA and RNA synthesis, protein synthesis (β-galactosidase), respiration, and oxidative phosphorylation are also inhibited in some species of bacteria and in rat liver homogenates (Morris and Russell, 1971), with none seemingly being the primary antibiotic effect. Novobiocin is also known to induce an intracellular magnesium deficiency, but there is no direct convincing evidence that this is the mechanism responsible for novobiocin's antimicrobial activity.

Novobiocin is initially active against *Staphylococcus* spp. infections, but resistance to this antibiotic develops quickly (Morris and Russell, 1971; Harvey, 1985). Novobiocin has been combined with tetracycline to produce synergistic activity, broaden the spectrum of activity and to decrease the resistance to novobiocin, but these older combinations are used infrequently today. Novobiocin and tetracycline have been reported to be efficacious in cases of canine upper respiratory diseases such as "kennel cough" and tonsillitis (Maxey, 1980), but the use of antibiotics for this problem in dogs has declined substantially. Toxic side effects in animals and humans given novobiocin systemically have been reported and include skin rashes, leucopenia, pancytopenia, anemia, agranulocytosis, thrombocytopenia, nausea, vomiting, and diarrhea. Few side effects have been reported for this antibiotic used in its topical form in domestic animals.

Thiostrepton

Thiostrepton is a polypeptide antibiotic produced by *Streptomyces aureus* and has a predominately gram-positive spectrum, although some gram-negative organisms are also affected. Thiostrepton is not absorbed from the gastrointestinal tract and is used primarily for topical local therapy, usually combined with other antibiotics and/or glucocorticosteroids for dermatological therapy.

Rifampin (Rifampicin)

Rifampin is an antibiotic, available for many years, that has been used in people to treat tuberculosis. Equine practitioners have been familiar with rifampin for many years because of its use for treating lung infections caused by *Rhodococcus equi*. Small animal veterinarians are becoming familiar with this antibiotic because it has appeared on susceptibility reports as being active against methicillin-resistant *Staphylococcus* spp.

This antibiotic was originally discovered in the pine forests of France in the 1950s and was introduced into clinical medicine in the 1960s. It is a complex macrocyclic high-molecular-weight semisynthetic antibiotic derived from rifamycin B, produced by *Nocardia mediterrea*. Rifamycin B is chemically modified to produce rifampin. Rifampin is the United States Pharmacopeia (USP) official name, and rifampicin is the International Nonproprietary Name (INN) and British Approved Name (BAN) name; both names are synonymous. Rifamycin and rifabutin are structurally similar antibiotics – all in the group of rifamycins – but are not identical.

Mechanism of Action and Spectrum

Rifampin is a bactericidal antibiotic that acts by inhibiting bacterial RNA polymerase. Rifampin enters the microbial cell and forms stable complexes with the β subunit of DNA-dependent RNA polymerases of microorganisms. This binding results in inactive enzymes and inhibition of RNA synthesis by preventing chain initiation. This inhibition can also occur in mammalian cells, but much higher concentrations are needed. MICs for gram-positive organisms generally occur at 0.1 μg/ml, while gram negative bacteria have MIC values ranging from 8 to 32 μg/ml. This large disparity in MIC values is attributed to rifampin's ability to more easily permeate the gram-positive organism cell wall than the gram-negative organism cell wall, rather than differences in bacterial RNA polymerases. The PK/PD parameter that best predicts rifampin efficacy is the AUC/MIC ratio.

Rifampin is highly lipophilic and the intracellular penetration has made this drug valuable for treating intracellular bacteria in people and animals, including *Mycobacterium* (tuberculosis), *Staphylococcus* spp., and *Rhodococcus equi*. Rifampin has activity against gram-positive bacteria (*Staphylococcus* spp.), *Mycobacterium* spp., *Haemophilus* spp., *Neisseria* spp., and *Chlamydia* spp., but more limited activity against the gram-negative bacteria. Rifampin is active against most strains of methicillin-resistant *Staphylococcus pseudintermedius* (Perreten et al., 2010) although resistance among canine isolates has been identified (Kadlec et al., 2011).

A single mutation of the amino acid sequence of the β subunit of the DNA-dependent RNA polymerase enzyme produces resistance. Mutations result in rifampin having less affinity for the RNA polymerase enzyme. After mutations arise, clonal spread of a resistant strain may occur.

For some infections, resistance can be minimized if other antibiotics are used concurrently that will kill the mutant strains of bacteria produced in response to rifampin. The study by Berghaus et al. (2013) showed that the mutant prevention concentration (MPC) is lower when rifampin is combined with macrolide antibiotics against *Rhodococcus equi.* To reduce the rate of mutation, combination therapy with other agents has been recommended in human guidelines (Liu et al., 2011) and was the recommendation from a veterinary study for treatment of *Staphylococcus* infections in dogs (Kadlec et al., 2011). For other bacterial infections, the combination of rifampin with other antibiotics may not produce a synergistic effect (Forrest and Tamura, 2010). Whether or not combination therapy is needed for veterinary use is discussed in more detail in Section Clinical Use. There is some evidence for a synergistic effect between amphotericin B and rifampin against some fungi, particularly *Saccharomyces cerevisiae, Histoplasma capsulatum,* several species of *Aspergillus,* and *Blastomyces dermatitidis* (Medoff, 1983). However, rifampin is rarely considered for treatment of a fungal infection because other effective antifungal drugs have emerged (see Chapter 38).

Pharmacokinetics

Pharmacokinetic features of rifampin in several species are presented in Table 36.10. Rifampin is lipophilic with a large volume of distribution and good absorption in practically all animal species studied. The oral absorption is moderate to high, ranging from 38–48% in foals to 70% in adult horses (Table 36.10). In sheep the oral absorption is 16–37%. It has even been absorbed from oral and rectal administration to elephants (Egelund et al., 2015). Rifampin absorption is highest in an acidic environment, although feeding has decreased oral absorption in foals and ruminants. Rifampin is approximately 80–85% bound to plasma proteins in people and 94% in foals. Despite high protein binding, it is widely distributed to all tissues of the body, with particularly high concentrations of the drug found in the lungs, pulmonary epithelial lining fluid, liver, bile, and urine. After oral absorption or parenteral administration, rifampin is primarily metabolized to the bioactive metabolite 25-desacetyl rifampin (25-*O*-desacetyl rifampin), which is active microbiologically. There are also some minor glucuronidation products formed in the liver. Both parent and metabolite compounds are excreted in the bile. Both forms are passively filtered through the kidneys, with renal clearance being approximately 12% of total glomerular filtration rate.

Multiple dosing of rifampin often results in decreased, rather than increased, peak serum concentrations. This phenomenon is due to autoinduction of liver enzymes and is known to occur in humans, swine, dogs, calves, horses, and rodents (Frank, 1990; Berlin et al., 2017). Hepatic enzyme induction and induction of efflux mechanisms by rifampin will also alter the disposition of other drugs (discussed in more detail in Section Interactions).

The half-life ranges from 11 to 14 hours in foals and the peak concentrations vary widely (Table 36.10). The rate of excretion in the foal is lower than in the adult horse, mainly due to biliary excretion mechanisms being less developed in the foal. In dogs the half-life is approximately 8 hours, with a peak concentration of 40 μg/ml.

Interactions

Rifampin is a potent activator of a transcription factor that increases the levels of many drug metabolizing proteins, including P-glycoprotein (P-gp), and cytochrome

Table 36.10 Pharmacokinetics of rifampin/ rifampicin in animals[a]

Species	Dose (mg/kg)	Half-life (hour)	Volume of distribution (Vd) (l/kg)[b]	AUC (μg h/ml)	Peak Concentration (C_{max}) (μg/ml)	Reference
Dogs	10 oral	8	–	–	35	–
	10 oral	5.84	–	42.3	7.4	Burrows et al., 1985[c]
	10 IV	6.05	0.635	120.2	2.9	Burrows et al., 1985[c]
	10 IV	7.27	0.932	118.57	–	Wilson et al., 1988[c]
Horses	20 oral	11.5	–	246.19	13.35	Wilson et al., 1988[c]
Foals	10 oral	14.7	–	160 (0–12 hours)	18.1	Peters et al., 2012
	10 oral	6.79	–	72.3	5.50	Berlin et al., 2017[d]
	20 oral	7.61	–	161	12.3	Berlin et al., 2017[d]
	10 IV	11.0	0.85	193	16.8	Berlin et al., 2017
	10 oral	11.5	–	77.0	8.2	Berlin et al., 2016
	10 IV	8.1	0.782	127.33	–	Kohn et al., 1993
	10 oral	–	–	67.65	3.86	Kohn et al., 1993
Calves[c]	10 oral	11.4	–	310.9	11.7–24.6	Sweeney et al., 1988
Calves[c]	10 IV					Sweeney et al., 1988
Sheep[c]	10 oral	4.3		11.7	0.6-2.4	Sweeney et al., 1988
Sheep[c]	10 IV	2.9	1.32	32	–	Sweeney et al., 1988
Sheep	20 oral	6.42	–		3.27	Jernigan et al., 1986
Sheep	20 IV	4.56	0.46	–	–	Jernigan et al., 1986

[a] Data represents a mean of the published values.

[b] Values for Vd represent Vd/F for oral doses.

[c] Concentrations measured using a microbiological assay, which can over-estimate the concentration because the metabolite is active.

[d] Data from Berlin et al., 2017; oral dose in foals was after repeated dosing for 10 days.

P-450 enzymes CYP3A, and CYP2C. The activation of occurs through an up-regulation of gene expression of intestinal and hepatic cytochrome P450 enzymes and transporters through the nuclear pregname X receptor (PXR) pathway (Reitman et al., 2011). The list of drugs identified in people that are affected by rifampin is long and summarized in other papers (Frank, 1990; Barriere et al., 1989; Lee et al., 1993; Reitman et al., 2011; Forrest and Tamura, 2010). The consequence of induction is a diminished effect of the coadministered drug and may require a higher dose or more frequent administration. When clarithromycin and rifampin were administered together in foals, it decreased the plasma drug concentration of clarithromycin by over 90% (Peters et al., 2011; Berlin et al., 2016). But in the same study, the concentration of rifampin was not affected by administration of clarithromycin. In people 4 weeks are required for full recovery of the rifampin effect after discontinuation (Reitman et al., 2011). Rifamin may have dual effects in which it can be an inhibitor of intestinal transport, as well as an inducer of other proteins.

Adverse Effects

Adverse effects have been associated with high doses and include liver injury and gastrointestinal disturbances. In a study of 344 dogs (Bajwa et al., 2013) adverse effects occurred in 16% of treated dogs. Adverse effects included vomiting, anorexia, lethargy, and weight loss. Gastrointestinal effects were the most common. There were 27% of dogs with increases in liver enzymes and this occurred between days 19 and 27 days of initiating treatment. Liver injury from rifampin is more common in dogs than in people or horses. It has been estimated that 20% or more of dogs receiving 5–10 mg/kg may develop increases in liver enzymes and some may develop hepatitis. It is advised to monitor hepatic enzymes during treatment in dogs and not to exceed a dose of 10 mg/kg per day. In the Bajwa study (Bajwa et al., 2013) cited above, a reduction in dose resolved adverse effects in many dogs. Rifampin has an unpalatable taste. It also may produce a reversible discoloration (orange–red color) to the urine, tears, and sclera. Pet owners should be warned of this possibility. Rifampin is teratogenic in laboratory animals, so its use in pregnant animals should be restricted.

Clinical Use

Susceptible organisms of interest to veterinarians include *Staphylococcus* species (including methicillin-resistant strains), *Streptococcus* spp., including *Streptococcus zooepidemicus*, *Rhodococcus equi*, *Corynebacterium pseudotuberculosis*, and most strains of *Bacteroides* spp., *Clostridium* spp., *Neisseria* spp., and *Listeria* spp. Organisms known to be resistant to rifampin are *Pseudomonas aeruginosa, E. coli, Enterobacter* spp., *Klebsiella pneumoniae*, *Proteus* spp., and *Salmonella* spp. Some gram-negative organisms may be susceptible, but it may require higher concentrations. Breakpoints for susceptibility testing of isolates from animals have not been established and the human breakpoint of ≤1, 2, and ≥4 µg/ml for susceptible, intermediate, and resistant, respectively, can be used until veterinary breakpoints are established by CLSI.

Rifampin has been used for treatment of gram-positive cocci infections in dogs (and occasionally cats), particularly methicillin-resistant *Staphylococcus* spp. that are resistant to other drugs. Rifampin has been effective for treatment of canine pyoderma caused by *Staphylococcus pseudintermedius* at a dose of 5 mg/kg once daily for 10 days (Sentürk et al., 2005).

Veterinary surgeons have recommended the addition of rifampin to treatment when biofilms are suspected to occur from surgical implants or chronic infections. Rifampin achieves high concentrations within neutrophils, endothelial cells, macrophages, and biofilms and, in people, has improved activity in combination with another antibiotic compared to that if it is used alone (Forrest and Tamura, 2010). No studies on biofilm infections have been reported in veterinary medicine.

There is a long history of rifampin use in horses. Rifampin is one of the first choices for treatment of infections in foals caused by *Rhodococcus equi* (Giguère et al., 2011). The dose for foals is typically 5 mg/kg oral, every 12 hours, but 10 mg/kg once per day also has been shown to attain effective concentrations (Berlin et al., 2017). It is routinely administered with one of the macrolide antibiotics – erythromycin, azithromycin, or clarithromycin (these drugs are discussed in Section Macrolide Antibiotics). In one uncontrolled retrospective study (Giguère et al., 2004) rifampin–clarithromycin combination was more effective than either rifampin–azithromycin or rifampin–erythromycin. In another study rifampin–azithromycin was more effective in foals than injections of tulathromycin (Rutenberg et al., 2017). Nevertheless, azithromycin is often used instead of clarithromycin because it is more convenient to administer.

Although not used frequently in ruminants, the pharmacokinetics have been studied (Table 36.10) and the recommended dose was 20 mg/kg orally once a day. The most common use in ruminants is for treatment of *Mycobacterium paratuberculosis* in cattle and sheep. It may cause remission of the infection, but does not eradicate the organism. It has also been used to treat *Mycobacterium tuberculosis* in elephants (10 mg/kg per day) (Egelund et al., 2015).

Monotherapy or combination therapy? Rifampin has been combined with other antimicrobials for treatment of *Staphylococcus* infections in dogs, and for treatment of *Rhodococcus equi* infections in foals in some protocols.

The reason for combination treatment for *Staphylococcus* infections in dogs is ostensibly to reduce emergence of resistance. However, there are no clinical studies in veterinary medicine that have demonstrated greater emergence of resistance from monotherapy with rifampin compared to combination therapy in dogs. The study by Sentürk et al. (2005) showed that monotherapy for *Staphylococcus* treatment in dogs was successful. In experimental infections rifampin monotherapy successfully eradicated staphylococci from pus in vitro and from abscesses in experimental infections (Lobo and Mandell, 1972).

The study by Kadlec et al. (Kadlec et al., 2011; Perreten et al., 2010) showed that rifampin resistance among staphylococci is infrequent. When treating staphylococcal infections in people (Falagas et al., 2007) addition of a second antibiotic did not confer additional effectiveness compared to rifampicin monotherapy for eradication of methicillin-resistant *Staphylococcus*. The study by Achermann et al. (2013) identified risk factors that contributed to resistance in staphylococcal joint infections. Rifampin monotherapy was not a statistical risk factor for development of resistance. This suggests that resistance to rifampin is possible with or without combination therapy.

The recommendation to use rifampin in combination with other antimicrobials to decrease emergence of resistance has been mainly validated in clinical situations in which long-term therapy with rifampicin was necessary (e.g., tuberculosis) and may not be the same for short-term treatment of *Staphylococcus.* Forrest and Tamura (2010) provided an extensive review of the use of rifampin in nonmycobacterial infections. They concluded that combinations of other drugs with rifampin results in indifference or antagonism and there are few examples showing synergism. For example in the treatment of staphylococcal infections, this analysis indicated that, “With a review period covering several decades, the in vitro data for rifampin combination therapy against staphylococci appear to frequently show antagonism or indifference, with synergy being found inconsistently.” This questions the benefit of adding other antibiotics to rifampin therapy for nonmycobacterial infections.

For the treatment of foals with *Rhodococcus equi* infection, rifampin has been combined with macrolide antibiotics – erythromycin, azithromycin, or clarithromycin most commonly. The recommendation of combination treatment of foals comes from consensus statements from experts (Giguère et al., 2011) and pharmacokinetic studies (Berlin et al., 2017). In pharmacokinetic studies, the concentrations in foal pulmonary epithelial lining fluid and bronchoalveolar lavage cells produced concentrations slightly lower than plasma concentrations but above the MIC_{90} for *Rhodococcus equi* (Berlin et al., 2017). It is possible that single-agent treatment is also effective, but this has not been compared with randomized, controlled studies. There is no evidence that combination treatments are synergistic using in vitro time-kill kinetic methods at achievable serum concentrations (Nordmann and Ronco, 1992), although combination with rifampin lowered the mutant prevention concentration (MPC) in vitro for some macrolides (Berghaus et al., 2012). The effects of rifampin on the pharmacokinetics of other antibiotics was discussed above in the Interactions section.

Nitrofurans

Nitrofurans comprise several synthetic compounds derived from 5-nitrofuran and possess antimicrobial activity, the 5-nitro group being required for this activity. Over 3,500 nitrofurans have been synthesized to date, with only a handful being useful in animal chemotherapy. Nitrofurans and furazolidone are banned from use in food-producing animals.

Nitrofurantoin is the main drug in this group administered orally. The mechanism of action is not well understood. After penetrating bacteria intracellular nitroreductases convert the drug to an active form through reduction of the nitro group. This action produces intermediate metabolites that bind to bacterial ribosomes and inhibit bacterial enzymes responsible for DNA and RNA synthesis.

The spectrum includes *E coli, Staphylococcus* spp., and *Enterococcus* spp. Resistance among bacteria is unusual, although *Proteus* and *Pseudomonas aeruginosa* are inherently resistant. Nitrofurans can be administered orally or topically. Oral absorption is 80% in people, but unknown in animals. Absorption is enhanced when administered with food. Serum concentrations are almost undetectable, or very low and therapeutically active concentrations are achieved only in urine. Effective concentrations cannot be achieved in the prostate or kidneys for treating upper urinary tract infection. An acid environment is required for the nitrofurans to diffuse across the cell membranes. Acidification of the urine promotes tubular reabsorption, which decreases the overall urine concentration of the drug.

Adverse Effects

The toxicology of furazolidone (*N*-5-nitro-2-furfurylidene amino-2-oxazolidinone) has been investigated extensively in laboratory, food, and companion animals as well as in humans, and has been reviewed by Ali (1989). The effects of feeding furazolidone to poultry have been reported (Ali, 1989; Mustafa et al., 1975; Czarnecki et al., 1974a, 1974b; Jankus et al., 1972).

Furazolidone has been demonstrated to be carcinogenic when used at a 0.15% w/w concentration in feed for 1 year, inducing mammary tumors in a dose-related manner. Mice fed a 0.03% w/w concentration in feed for life developed bronchial adenocarcinomas in both sexes (Ali, 1983). DNA is the principal target of furazolidone in some cells in vivo, causing cuts and mutations in DNA and binding to DNA, hence blocking the replication and transcription processes. Mutagenesis by nitrofurans in general also occurs and has been extensively reviewed by McCalla (1983), who notes several possible metabolic pathways by which nitrofurans can cause mutagenesis in mammalian cells. This potential for mutagenesis and carcinogenesis has caused the ban from use in food-producing animals.

The major disadvantage of nitrofurans to treat systemic infections is that the concentrations needed to reach the MIC also induce systemic toxicity. There are early reports in the veterinary literature on the toxicities induced when the nitrofurans are used systemically (Ali, 1983). Oral nitrofurantoin adverse effects include nausea, vomiting, and diarrhea. It turns urine rust-yellow brown color. In people, respiratory problems (pneumonitis) and peripheral neuropathy have been reported. The polyneuropathies in people are caused by a demyelination and are more common from long-term use or in patients with renal compromise. The respiratory problems have not been reported in animals, but neuropathy has been observed in dogs; the risk may be higher if there is renal insufficiency.

Clinical Use

Except for topical use, the most frequent use of nitrofurantoin in veterinary medicine is for treatment and prevention of urinary tract infections in dogs and cats that are resistant to other antimicrobials. The oral forms (Macrodantin, Furalan, Furatoin, Furadantin, and generic brands) have been administered to dogs for urinary tract infections when there are other options are limited. Although this use has been incorporated into some urinary tract infection protocols for dogs and cats, the efficacy is undetermined. Typical doses are 10 mg/kg/day divided into four daily treatments, then reduced to 1 mg/kg per day. It does not attain high enough concentrations for other infections. Although oral absorption has not been studied in dogs, in people the macrocrystalline form is slowly absorbed and less likely to cause gastric upset. The microcrystalline form is rapidly absorbed in intestine.

Although efficacy has not been examined in animals, a metaanalysis of human studies showed efficacy for short-term treatment of uncomplicated urinary tract infections (Huttner et al., 2015). Their analysis included a review of clinical trials from 1946 to 2014. They concluded that there was overall equivalence between nitrofurantoin when given for 5 or 7 days and trimethoprim–sulfamethoxazole, fluoroquinolones, and amoxicillin. Adverse effects in people were uncommon if treatment duration was kept short. The most serious adverse effects of pulmonary fibrosis and liver injury are the result of administration for several months or years.

Virginiamycin

Virginiamycin is a combination of two chemicals produced by *Streptomyces virginiae*, isolated from soil samples in Belgium in the early 1960s. Virginiamycin is classified as a peptolide antibiotic composed of the predominate M fraction ($C_{28}H_{35}N_3O_7$) and the lesser S fraction ($C_{43}H_{49}NO_{10}$) (Crawford, 1984). The optimum ratio of M : S is 4 : 1 (Gottschall et al., 1988). Administered separately, both M and S fractions have a reversible bacteriostatic action on susceptible bacterial populations; used together, their activity is synergistic, bactericidal, and approximately 100 times that found when used separately. Virginiamycin is not known to be synergistic with other classes of antibiotics. Virginiamycin is primarily active against gram-positive organisms, *Haemophilus* spp., and *Neisseria* spp., and has mild activity against the protozoan *Toxoplasma* spp. It works by inhibiting protein synthesis at the 23S ribosomal subunit, blocking translation but not transcription in susceptible bacteria. Virginiamycin is rapidly absorbed when administered orally, is excreted by the bile with no enterohepatic circulation, and has an affinity for dermal tissues (Crawford, 1984). Gottschall et al. (1988) reported that ^{14}C-virginiamycin, specifically the M fraction, was extensively metabolized in the rumen. The S fraction underwent no detectable metabolism in the rumen, and the M fraction metabolites had considerably less antimicrobial activity than the parent compound.

All virginiamycin-like antibiotics fall into one of two groups. Group A consists of polyunsaturated cyclic peptolides that have a molecular weight of approximately 500 and that contain substituted aminodecanoic acid and an oxanzole system. Group B consists of cyclic hexadepsipeptides with an approximate molecular weight of 800, and most members contain one molecule of pipecolic acid or its derivative. Both groups have low solubilities in aqueous solvents and are more soluble in organic solvents. All strongly absorb ultraviolet radiation and are therefore degraded in its presence. Virginiamycin-like antibiotics tend to affect gram-positive bacteria more than the gram negative, with *Mycobacteria* spp. being relatively resistant and *Haemophilus* spp. and *Neisseria* spp. being very sensitive. Differences in bacterial sensitivity to different virginiamycin-like antibiotics are caused by each antibiotic's particular ability to permeate that bacteria's cell wall to gain access to the ribosomes (Cocito, 1979).

Virginiamycin and virginiamycin-like antibiotics are not commonly used to treat clinical bacterial disease in domestic animals, despite their rather broad spectrum of activity. They have been used to treat swine dysentery (*Treponema hyodysenteriae*) (Olsen and Rodabaugh, 1977), but other antibiotics have proven to be more efficacious. Its main use has been as a feed additive for growth promotion in food animals such as swine (Ravindran and Kornegay, 1984; Moser et al., 1985), being approved for this use since 1975, but use of antibiotics for growth promotion is being phased out in most countries. Virginiamycin has also been studied in turkeys (Salmon and Stevens, 1990), broilers (Miles et al., 1984), and laying hens (Miles et al., 1985) as a growth promotant, all of which experienced either increased weight gain or increased egg production. The regulation and use of antibiotics used in feed for food producing animals is discussed more in other chapters (Chapters 52 and 59).

Carbadox

Carbadox (methyl 3-(2-quinoxalinylmethylene) carbazate N_1,N_4 dioxide) is a synthetic antibacterial agent primarily active against the gram-positive bacteria, although some gram-negative bacteria are affected as well. Carbadox is an antibiotic used in swine (hogs and pigs) for production purposes (e.g., increased rate of weight gain and improved feed efficiency) and therapeutic purposes (e.g., to control swine dysentery and bacterial swine enteritis). While carbadox is an antimicrobial, it does not pose the same resistance issues as other antimicrobials and is not considered important to human medicine.

Available in 1973, carbadox was marketed as a growth promotant in swine and also for the control of swine dysentery (*Treponema hyodysenteriae*), bacterial enteritis (in particular, *Salmonella cholerasuis*), and nasal infections of *Bordetella bronchiseptica* in swine (Farrington and Shively, 1979). Carbadox was shown to be better than lincomycin for the treatment of swine dysentery (Anonymous, 1980; Rainier et al., 1980). Resistance to carbadox has been reported in *E. coli* via R-plasmids (Ohmae et al., 1981). There are three approved New Animal Drug Applications (NADAs) for animal drug products containing carbadox: a premix with carbadox alone, carbadox plus pyrantel tartrate, and carbadox plus oxytetracycline.

The daily feeding of carbadox in feed concentrations of more than 100 ppm for growth promotion in pigs has resulted in toxicities in some weaned pigs, which include growth retardation, dry feces, wasting, dehydration, urine drinking, and a strong interest in salt-containing products (van der Molen et al., 1989a). It is now known that carbadox suppresses aldosterone production, leading to hypoaldosteronism, which then leads to decreased plasma sodium and increased plasma potassium concentrations. These ion alterations are due to stimulation of the renin–angiotensin system with subsequent morphological changes in the zona glomerulosa of the adrenal cortex (van der Molen et al., 1989a, 1989b, 1989c).

Regulatory actions: The United States FDA Center for Veterinary Medicine (CVM) has questions about the safety of carbadox. On April 8, 2016, the FDA announced a proposal to withdraw the approval of the drug applications for products containing carbadox. In addition, the use of antimicrobial agents for growth production in farm animals is being phased out in most countries. Regulation of food-animal antibiotics is discussed in greater detail in other chapters (Chapters 52 and 59).

Glycopeptides (Vancomycin)

Of the glycopeptides, vancomycin is the only one used in veterinary medicine. Teicoplanin has been used in Europe but is not available in the United States. Use of vancomycin in veterinary medicine is limited, but has been necessary in some cases for resistant enterococcal or staphylococcal infections. In August 20, 1997 the US Food and Drug Administration prohibited the extralabel use of glycopeptides in food-producing animals. The reason for this action is because of a fear of glycopeptide-resistant bacteria transmitted to people from treated animals (Bates et al., 1994). This action emerged from the association between resistant enterococci and the use of glycopeptides such as avoparcin and ardacin in animal feed in Europe (Bates et al., 1994). Use of these glycopeptides in animal feeds has been discontinued.

Over 80 *n*-alkyl vancomycins have been synthesized by reductive alkylation of vancomycin, with some forms being five times more active than vancomycin and with some having longer elimination half-lives (Nagarajan et al., 1989). There are new drugs related to vancomycin that have been added to human treatment, but their use of these has not been reported in animals. These drugs include dalbavancin, oritavancin, and telavancin. Telavancin (Vibativ) is a lipoglycopeptide, for once-daily administration. Dalbavancin (Dalvance) is a long-acting IV lipoglycopeptide, similar to telavancin (Vibativ). The half-life in people is 14 days, which allows for once/week treatment. Oritavancin (Orbativ) is a long-acting IV lipoglycopeptide, also similar to telavancin. The half-life is approximately 14 days, which allows for a single treatment.

Vancomycin was discovered in the 1950s. In the 1960s and 1970s it was not used much because the penicillins and cephalosporins were active against most gram-positive bacteria. But in the last 10–15 years drug-resistant enterococcal and staphylococcal infections have generated more reliance on vancomycin in human medicine. Vancomycin is a tricyclic glycopeptide having an approximate molecular weight of 1500.

It is produced by the soil-borne actinomycete *Streptomyces orientalis*. It is freely soluble in water, odorless, and slightly bitter to the taste. Vancomycin inhibits the transpeptidase and transglycosylase steps in bacterial cell-wall synthesis of gram-positive bacteria by binding to the terminal D-alanyl-D-alanine of the stem pentapeptide (cell wall precursor) of the nascent peptidoglycan. Vancomycin is bactericidal for most organisms and bacteriostatic for enterococci. The bactericidal action occurs by activating bacterial cell wall autolysins. This action occurs slowly and there is a gradual loss of cell wall integrity that may not have full effects until 24 hours. The action is time dependent, but the PK-PD that best predicts clinical results is the area-under-the-curve (AUC) to MIC ratio (AUC : MIC) with a target of > 400 considered ideal (Vandecasteele et al., 2013).

Vancomycin is highly active against gram-positive cocci (in particular, *Staphylococcus* spp. and streptococci), enterococci (*Enterococcus faecium* and *E. faecalis*), as well as *Neisseria* spp. Because it has activity against methicillin-resistant *Staphylococcus* species, including *Staphylococcus aureus* (MRSA) and *Staphylococcus pseudintermedius* (MRSP) and β-lactam resistant *Enterococcus* species, it is valuable for the treatment of these infections. It also is active against gram-positive anaerobic cocci (but not anaerobic gram-negative bacteria) and has been administered to people for diarrhea caused by *Clostridium* spp.

Adverse Effects

Toxicity studies on vancomycin have been performed in many species of laboratory animals (Wold and Turnipseed, 1981). The LD_{50} for the canine was 292 mg/kg, but death did not occur until several days after dosing. Dogs died because of kidney injury, with death due to acute nephrotoxic renal failure.

If vancomycin is administered according to the recommended dosing rates, adverse reactions described earlier are uncommon. A slow infusion is recommended to minimize histamine release. To avoid other toxic reactions, dose recommendations are designed to avoid high plasma concentrations. In people, therapeutic drug monitoring is often performed to ensure that peak concentrations are below 50 μg/ml. The current recommendations are for trough serum vancomycin concentrations of 15–20 μg/ml for intermittent dosing and plateau serum vancomycin concentrations of 20–25 μg/ml for continuous infusions. If animals have renal disease or unique physiological changes (e.g., pregnant or a neonatal animal), drug disposition may change, and peak and trough plasma concentrations should be monitored to adjust the dose appropriately.

Early formulations of vancomycin were associated with a high incidence of adverse effects. Most of these effects were associated with rapid IV administration, which induced flushing of the skin, pruritus, tachycardia, and other signs attributed to histamine release. Ototoxicity also was reported. Kidney injury risk is greater with high doses and longer exposure. Vancomycin toxicity acts as an oxidative stressor in the renal proximal tubule and can produce interstitial nephritis. The incidence of nephrotoxicity and ototoxicity may be partially caused by the common practice of simultaneously administering vancomycin with aminoglycosides. Newer and higher quality formulations of vancomycin have avoided some of the most serious adverse events, but histamine release still is possible from IV administration.

Clinical Use and Administration Guidelines

Clinical use of vancomycin has been limited in veterinary medicine and most of our clinical recommendations for use are derived from pharmacokinetic studies performed in dogs and horses and recommendations of effective blood concentrations for people. Vancomycin must be administered via IV infusion, although in rare instances intraperitoneal administration has been used. Vancomycin is poorly absorbed orally and this route is not used except to treat intestinal infections. IM administration is painful and irritating.

In dogs the half-life is somewhat shorter and the volume of distribution smaller than in humans (Zaghlol and Brown, 1988). In people, the suggested trough concentration is 15–20 μg/ml, but it is difficult to maintain these concentrations in dogs because of the short half-life. A dose rate of 15 mg/kg q 6–8 h IV actually produces peaks and troughs of approximately 40 and 5 μg/ml, respectively, but it is the most convenient dose that can be used because of the short half-life in dogs. This dose should be infused slowly over 30–60 minutes, or at a rate of approximately 10 mg/min. The total dose to be administered can be diluted in 0.9% saline or 5% dextrose solution, but not alkalinizing solutions. Vancomycin is available in vials of 500 mg to 5 g (Vancocin, other brands, and generic). If vancomycin is used to treat enterococcal infections, it is strongly recommended to coadminister an aminoglycoside (e.g., amikacin or gentamicin) because when used alone, vancomycin is not bactericidal.

Vancomycin is used infrequently in horses, but has been necessary occasionally for treatment of methicillin-resistant *Staphylococcus* infections and drug-resistant infections caused by *Enterococcus*. The guidelines for treatment were developed by Orsini et al. (2005) from their pharmacokinetic studies. A dose of 7.5 mg/kg is infused over at least 30 minutes every 8 hours in horses. Adverse reactions with this protocol have been minimal. To treat distal limb infections in horses regional limb perfusion or interosseous regional infusions have been used according to a protocol developed by Rubio-Martinez et al. (2005, 2006), in which 300 mg total dose is diluted in 60 ml saline solution and infused in the distal limb.

Methenamine

Methenamine (hexamethylenetetramine) is a urinary antiseptic most commonly used to treat urinary tract infections in small animals. It may be used in conjunction with an antibiotic or occasionally by itself in some cases of bacterial urinary tract infections that have become refractory to conventional antibiotic therapies. Methenamine is activated by a hydrolysis reaction to form formaldehyde and ammonia in acidic urine. It may be effective against a wide variety of gram-positive and gram-negative organisms. It can be either bacteriostatic or bactericidal depending on the pH of the urine (Harvey, 1985).

Methenamine is quickly absorbed when given orally, but absorption is not complete because some is hydrolyzed in the stomach. It is excreted via the urine, and is associated with a low systemic toxicity.

The urine must be at an acidic pH in order to liberate free formaldehyde; therefore, methenamine is most effective when the urine pH is 6 or below. One of the forms of methenamine used is methenamine hippurate, which is available in human tablet form and is administered to dogs at a dose extrapolated from people of 500 mg per dog, oral, every 12 hours. Another form, methenamine mandelate is no longer available commercially. Methenamine has also been administered with urinary acidifiers to lower the urine pH. Concurrent use of other urinary acidifiers, ascorbic acid, arginine HCl, methionine, cranberry juice, and ammonium chloride, may enhance the antibacterial action of methenamine because acidic urine also exerts some independent weak antibacterial activity. Sulfonamides should not be administered with methenamine due to the formation of insoluble formaldehyde–sulfonamide precipitates. Since methenamine is largely eliminated via the kidney, its use should be restricted or closely monitored in cases of renal insufficiency (Harvey, 1985). Methenamine is less effective for treating infections caused by urea-splitting organisms, which increase the urine pH (e.g., *Proteus mirabilis*).

Methenamine mandelate has been used experimentally in the treatment of burn wounds in rats. Topical doses of 5% and 10% were highly efficacious against experimentally induced burns infected with a virulent strain of *Pseudomonas* spp. (Taylor et al., 1970).

Polymyxins

Polymyxins are a group of *N*-monoacetylated decapeptides discovered in 1947 and are produced by *Bacillus polymyxa*. They contain seven amino acids in a cyclic configuration and have a molecular weight of approximately 1000. Several polymyxins have been isolated and have been named A, B, C, D, E, and M. Of these six antibiotics, B and E in their sulfate salt forms are the only ones used clinically. Polymyxin B1 has a pK_a ranging from 8 to 9. The largest use of polymyxin has been in topical ointment preparations. Polymyxin B sulfate is a mixture of polymyxin B1 ($C_{56}H_{98}N_{16}O_3$) and polymyxin B2 ($C_{55}H_{96}N_{16}O_{13}$); polymyxin E is more commonly known as colistin (Harvey, 1985). Colistin has seen a resurgence for treatment of carbapenem-resistant Enterobacteriaceae (CRE) in people for which few other treatment options exist. This use has not been examined in veterinary medicine.

Polymyxins are basic surface-active cationic detergents that interact with the phospholipid within the cell membrane, penetrate that membrane, and then disrupt its structure. This action subsequently induces permeability changes within the cell that result in cell death, giving polymyxins bactericidal properties.

Polymyxins are not absorbed to any extent from the gastrointestinal tract when administered orally. Administration is by injection, usually IV. After administration polymyxin B is 70–90% plasma protein binding and distributed to the heart, lungs, liver, kidney, and skeletal muscle, with excretion mainly via the urine (Sande and Mandell, 1990a). The pharmacokinetics of some of the polymyxins in calves, ewes, rabbits, and dogs is reviewed in greater detail elsewhere (Ziv and Sulman, 1973a; Ziv et al., 1982; Craig and Kunin, 1973; al-Khayyat and Aronson, 1973a, 1973b). Ziv and Sulman (1973a) reported that an IV administration of 5 mg/kg polymyxin B in ewes resulted in a serum half-life of 2.7–4.3 hours and a Vd of 1.29 l/kg.

Polymyxins have a gram-negative antibacterial spectrum, which includes species of *Aerobacter, Escherichia, Histophilus, Klebsiella, Pasteurella, Pseudomonas, Salmonella,* and *Shigella. Proteus* spp. and most strains of *Serratia* spp. are not affected by polymyxins, and all gram-positive bacteria are resistant. If bacteria are sensitive to the polymyxins, they rarely acquire resistance.

Since the polymyxins are not absorbed into the body when given orally, polymyxin B has been used for “bowel sterilization” prior to abdominal surgeries and in irrigation solutions to flush the peritoneal cavities during those procedures. Polymyxins used to be the major drugs for treatment of *Pseudomonas* infections in humans, but since the advent of better penicillins, aminoglycosides, and cephalosporins, their use has declined over time. Nephrotoxicity occurs due to glomerulus and tubular epithelium damage. In addition, respiratory paralysis (usually caused by a rapid IV injection, too much peritoneal lavage, or a preexisting renal condition) and CNS dysfunction, including depression, pyrexia, and anorexia, also occur.

Polymyxins are mainly used in topical skin, mucous membrane, eye, and ear preparations. One of the most common “first-aid” antibiotics is the *Triple Antibiotic* containing bacitracin zinc, neomycin sulfate, and polymyxin B sulfate. Typically, one gram of ointment contains 400 units bacitracin zinc, 3.5 mg neomycin sulfate, and 5,000 units polymyxin B sulfate 5,000. No adverse systemic effects have been reported when they are applied to intact or denuded skin surfaces. Polymyxin antibacterial activity is markedly decreased in the presence of pus, in tissues containing acidic phospholipids, divalent cations, unsaturated fatty acids, debris, purulent exudate, quaternary ammonium compounds, and in the presence of anionic detergents or other chemicals that antagonize cationic detergents (Harvey, 1985).

In addition to its narrow-spectrum antimicrobial properties, polymyxin B has demonstrated a protective effect against the adverse effects of endotoxin produced by gram-negative bacteria. Polymyxin is capable of acting as a chelating agent to bind the lipid A portion of endotoxin in a 1 : 1 ratio to neutralize lipopolysaccharide. This effectively renders the endotoxin inactive, thereby preventing most of the adverse effects of gram-negative endotoxin. This property has been studied more in horses than in other species and has been part of an antiendotoxin protocol in equine medicine (Morresey and Mackay, 2006). Infusions ranging from 1,000 to 10,000 units per kg (a common dose is 6,000 units/kg, equivalent to 1 mg/kg) administered every 8 hours have been shown to be safe and effective for treating endotoxemia in horses.

Polymyxin B is available in vials containing 500,000 polymyxin B units. In some dosage protocols, the dose is listed in milligrams instead of units. One milligram of polymyxin B base is equivalent to 10,000 units of polymyxin B, and each microgram of pure polymyxin B base is equivalent to 10 units of polymyxin B.

Spectinomycin

Spectinomycin (Spectam) resembles aminoglycosides in some properties. It is highly water soluble and is easily mixed in aqueous solutions. Compared to aminoglycosides, it does not contain amino sugars or glycosidic bonds, but it has an aminocyclitol nucleus like the aminoglycosides. An important difference between spectinomycin and the aminoglycosides is in the adverse effects and spectrum of activity. Spectinomycin lacks the toxic effects of aminoglycoside antibiotics and can be used without concerns about kidney injury. Most formulations of spectinomycin have been removed from the commercial market. The combination products for livestock (for example, lincymycin–spectinomycin) have been discontinued in some countries.

Antimicrobial Activity

Spectinomycin, like aminoglycosides inhibits protein synthesis via a 30S ribosomal target. It is a broad-spectrum drug with activity against gram-positive and some gram-negative bacteria, but little anaerobic activity. It also has good activity against *Mycoplasma*. It is used in cattle because it has activity against *Mannheimia (Pasteurella) haemolytica, Pasteurella multocida,* and *Histophilus somni* (formerly *Haemohilus somnus).*

Pharmacokinetics

Like the aminoglycosides, spectinomycin has a small volume of distribution. It is poorly absorbed after oral administration (10% or less), but some systemic levels are achieved after oral administration in monogastric animals. Most of an oral dose is eliminated in the feces, but after an injection the primary route of elimination is the kidneys. Oral administration has been used for a local effect for treatment of diarrhea.

The half-life of spectinomycin is approximately 2 hours in cattle at a dose of 10 mg/kg IM or IV, and 1.2 hours after a dose of 20 mg/kg IV. After oral dosing in dogs of 100 or 500 mg/kg, the plasma concentrations (peak) were 22 and 80 µg/ml, respectively. The half-life in dogs is 2.72 hours at the 100 mg/kg dose. After IM injection in dogs, the half-life was 1.1 hours. In pigs, the half-life was 1 hour after IM administration.

Clinical Use

Older formulations have been used that contain both lincomycin and spectinomycin. Formulations containing only spectinomycin (Spectam) include spectinomycin oral solution, spectinomycin powder for drinking water, and spectinomycin hydrochloride injection for poultry. Spectinomycin sulfate has been used for injection in cattle (Adspec®), which is more highly absorbed than the hydrochloride salt. Spectinomycin tablets for dogs are an old formulation that is not currently used. (The sponsor of Adspec®, Zoetis, has announced discontinuation of the manufacture of this drug.) There are no small animal formulations currently available but the old formulation for small animals was spectinomycin hydrochloride pentahydrate administered as tablets (22 mg/kg twice daily) or by injection (5–10 mg/kg) for treatment of nonspecific infections.

Clinical use is primarily confined to food animals. Administration to horses has not been reported. In pigs, spectinomycin has been used orally as spectinomycin hydrochloride oral solution. It has in vitro activity against some gram-negative bacteria and has been used as a feed additive for pigs because of its activity against *Mycoplasma*. It has also been administered orally to pigs for treatment of bacterial enteritis caused by *E. coli* (50–100 mg/animal, oral) and as an injection for treatment of respiratory infections. In poultry, spectinomycin has been used as injection and added to drinking water for prevention and treatment of respiratory disease and other infections.

In cattle, spectinomycin has been used as an IM injection for treatment of respiratory infections (10–15 mg/kg SC in the neck, q 24 h × 3–5 days). Poultry formulations were used in cattle off-label prior to approval of Adspec®, when it was a past practice to mix the water soluble powder intended for drinking water as an IV solution for administration to cattle. This use is discouraged because it may result in severe pulmonary edema and death.

Oxazolidinones

The oxazolidinones are infrequently used in veterinary medicine. The most common one used from this class is linezolid (Zyvox®), which was the first one introduced to human medicine. It is valuable for use in people to treat resistant gram-positive infections caused by enterococci and methicillin-resistant *Staphylococcus* spp. Because there are so few drugs active against methicillin-resistant *Staphylococcus* (MRSA), it is the only oral drug that is consistently effective for this infection in people and also used for drug-resistant *Enterococcus*. Because of the increase in the incidence of methicillin-resistant *Staphylococcus* in dogs and cats (methicillin-resistant *Staphylococcus pseudintermedius,* MRSP), the use of linezolid has been increasing in these animals. The brand name of this drug (Zyvox®) was almost prohibitively expensive, but the newer generic forms are one-tenth of the cost, or less, which has increased the use. There is a new oxazolidinone available for people, tedizolid (Sivextro®) which is available in tablets (200 mg) and IV. It has properties similar to linezolid and clinical uses similar in people.

Linezolid inhibits protein synthesis by binding to the bacterial ribosome. It is bacteriostatic by binding to a site on the bacterial 23S ribosomal RNA of the 50S subunit. This prevents formation of the 70S ribosomal unit, and therefore protein synthesis is inhibited. It has activity primarily against gram-positive bacteria, particularly staphylococci and enterococci. Linezolid has good penetration into cells and extracellular fluid. Urine concentrations are high enough to inhibit urinary tract pathogens. In dogs the pharmacokinetics are similar to humans. The oral absorption is almost 100%, with or without food, and the half-life is slightly faster than humans. Linezolid does not undergo hepatic P450 metabolism, and one-third of the total clearance relies on the kidneys.

Adverse effects

Adverse effects have not been reported from use in dogs or cats, but clinical use has been rare enough that this may not be detected. In people, nausea and diarrhea can occur. Long-term use can cause bone marrow suppression (e.g., thrombocytopenia) in people, but this has not been reported in dogs or cats. Rarely, anemia and leukopenia have been observed in people. The risk of bone marrow suppression is most evident after 2 weeks of treatment. Long treatment courses in animals should be accompanied by periodic CBC measurements until the risk of bone marrow suppression is better understood in these animals.

Drug Interactions

Linezolid is a Type-A monoamine oxidase inhibitor (MAOI). Possible interactions occur with serotonin reuptake inhibitors such as fluoxetine and selegiline to produce serotonin syndrome. Interactions are also possible if administered with adrenergic drugs such as phenylpropanolamine. However, this effect has not been reported for animals. Linezolid is not expected to affect metabolism of other drugs. Rifampin may decrease plasma concentrations. Intravenous formulation is physically incompatible with other drugs in IV line. If administered with other drugs IV, flush out the administration line first.

Clinical Use

The use of linezolid should be selected on the basis of susceptibility monitoring and it is not recommended for routine use in animals unless other options are not available because of drug resistance. CLSI lists the break point for susceptibility as less than or equal to 4.0 μg/ml for *Staphylococcus* spp. and less than or equal to 2.0 μg/ml for *Enterococcus*. Linezolid is available in 400- and 600-mg tablets, 20-mg/ml oral suspension powder, and 2-mg/ml injection. The tablets have been expensive, but recent introduction of generic forms has lowered this cost. A typical dose for dogs and cats is 10 mg/kg q 12 h oral or IV.

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37

Fluoroquinolone Antimicrobial Drugs

Mark G. Papich

The fluoroquinolone antibacterial agents are among the most important antimicrobials used in veterinary medicine. They are used in practically all species and have a broad spectrum of activity that includes most of the important veterinary bacterial pathogens. Since their first introduction in the late 1980s (discussed in previous editions of this book), the use of fluoroquinolones has expanded tremendously.

The fluoroquinolones are synthetic antibacterial agents introduced in veterinary medicine first as enrofloxacin. Since then, there has been a great deal of research on this class of drugs to better understand their mechanism of action, antimicrobial spectrum, pharmacokinetics in a wide variety of animal species, and clinical uses. In addition, pharmaceutical companies have developed new agents in this class to increase the number of drug choices available to veterinarians. The advantages of the fluoroquinolones are that they are rapidly bactericidal against a wide variety of clinically important bacterial organisms, are potent, are well tolerated by animals, and have been administered via a variety of routes (orally via tablets and drinking water, subcutaneously, intravenously, intramuscularly, and topically).

Fluoroquinolones approved for use in veterinary medicine for small animals are shown in Table 37.1. Fluoroquinolones that are approved for humans and are of potential interest for veterinary medicine include ciprofloxacin. The newest generation of fluoroquinolones, with increased activity against gram-positive cocci and anaerobic bacteria, includes levofloxacin, moxifloxacin, gatifloxacin, and the veterinary drug pradofloxacin.

Chemical Features

The currently available fluoroquinolones have the same quinolone structure (Figure 37.1). The addition of the fluorine group at position 6 gives these the characteristics of a fluoroquinolone, and various other chemical substitutions and side groups account for the different physical characteristics of each drug. Enrofloxacin has one fluorine substitution, difloxacin has two fluorine substitutions, and orbifloxacin has a three-fluorine substitution, but the presence of more than one fluorine does not increase antibacterial effects (Asuquo and Piddock, 1993). When lipid solubility is expressed as the octanol: water partition coefficient, enrofloxacin has among the highest lipophilicity. By comparison, ciprofloxacin has a partition coefficient that is approximately 100-fold less than that of enrofloxacin; the corresponding partition coefficients of orbifloxacin and marbofloxacin are slightly higher than that of ciprofloxacin (Asuquo and Piddock, 1993; Takács-Novák et al., 1992). But there are no studies available to show that these chemical differences among the drugs can account for differences in clinical response. However, chemical differences may account for some variation in absorption and distribution. For example, ciprofloxacin oral absorption is approximately one-half that of enrofloxacin in dogs; ciprofloxacin absorption in horses is less than 10%, compared to 60% for enrofloxacin. The less lipid-soluble fluoroquinolones (marbofloxacin, orbifloxacin) have a lower volume of distribution (Vd) than the ones with higher lipid solubility (enrofloxacin) (Table 37.2). One explanation for this observation is that the more lipid-soluble drugs have higher intracellular concentrations, but higher tissue binding also could explain the differences in volume of distribution.

The most important structural difference in recent years is the addition of a substitution at position 8. These are usually referred to as *third-generation* fluoroquinolones to distinguish them from the *second-generation* drugs such as ciprofloxacin and enrofloxacin. For some human drugs, the addition is a methoxy (moxifloxacin), and for one of the veterinary drugs, it is a cyano group at position 8 that confers increased spectrum of activity.

Quinolones are amphoteric molecules that can be protonated at the carboxyl and the tertiary amine portion of the molecule (Martinez et al., 2006). The pK_a varies

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Table 37.1 Examples of fluoroquinolones used in veterinary medicine

Drug	Brand name(s)	Approved species
Enrofloxacin	Baytril, and generic	Dogs, Cats, Cattle, Pigs
Danofloxacin	Advocin	Cattle
Orbifloxacin	Orbax	Dogs, Cats
Pradofloxacin	Veraflox	Cats, (also dogs in some countries)
Marbofloxacin	Zeniquin, Marbocyl	Dogs, Cats (other species outside the U.S.)
Ciprofloxacin	Cipro and generic	Not approved for animals; human drug
Ibafloxacin	Ibaflin	No longer available (formerly dogs, cats)
Difloxacin	Dicural	No longer marketed (formerly dogs, and in some countries, cattle, and poultry)

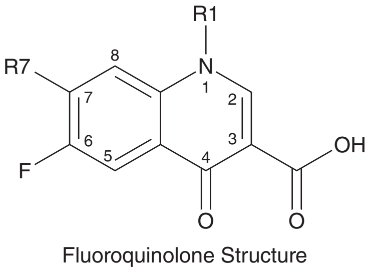

Figure 37.1 Structure of the fluoroquinolones. Features necessary for antibacterial activity are fluorine at position 6, ketone at position 4, and carboxyl at position 3. (Figure 37.2 shows other additions.) Addition of cyclopropyl, ethyl, or fluorophenyl at position 1 and of piperazine at position 7 increases the spectrum of antibacterial activity. Substitutions at position 8 broaden the spectrum of activity.

among the drugs slightly, but generally the pK_a for the carboxyl group is 6.0–6.4 (5.5–6.3 in some references) and the pK_a for the nitrogen of the piperazine group is 7.5–8 (Nikaido and Thanassi, 1993) (as high as 7.6–9.3 in some references). For two common drugs, enrofloxacin and ciprofloxacin, the pK_a for the carboxyl group is 6.0 and 6.1, respectively, and 8.8 and 8.7 for the amine, respectively (Martinez et al., 2006). The isoelectric point is midway between the pK_a for each ionizable group. Therefore, at physiological pH fluoroquinolones exist as zwitterions, in which both of the respective anionic and cationic groups are charged. It is at the isoelectric point (near the physiological pH) that fluoroquinolones are the most lipophilic and capable of diffusing across lipid membranes (Takács-Novák et al., 1992; Martinez et al., 2006).

Structure–Activity Relationships

Figure 37.1 shows the basic quinolone structure. The carboxyl group at position 3 and the ketone at position 4 are necessary for the antibacterial activity. The fluorine at position 6 differentiates the quinolones from the fluoroquinolones and accounts for the improved gram-negative and gram-positive activity over the nonfluorinated quinolones, increased potency, and increased entry into bacteria. At position 1, addition of a cyclopropyl (as for enrofloxacin and ciprofloxacin in Figure 37.2), an ethyl or a fluorophenyl improve the spectrum of activity against gram-positive and gram-negative bacteria. Addition of a piperazine at position 7, as demonstrated for ciprofloxacin, marbofloxacin, and enrofloxacin (Figure 37.2), improves the spectrum of activity to include pseudomonads, among other gram-negative bacteria. The change from a nitrogen (nalidixic acid) to a carbon at position 8 decreased some of the adverse central nervous system effects and increased activity against staphylococci.

Table 37.2 Interpretive categories for susceptibility break points (CLSI, 2015)

Drug	Species	Susceptible (μg/ml)	Intermediate (μg/ml)	Resistant (μg/ml)
Enrofloxacin	Dogs, cats	≤0.5	1–2	≥ 4
Orbifloxacin	Dogs, cats	≤1.0	2–4	≥ 8
Ciprofloxacin*	People	≤1.0	2	≥ 4
Marbofloxacin	Dogs, cats	≤1.0	2	≥ 4
Enrofloxacin	Pigs (gram negative)	≤0.25	0.5	≥ 1
Enrofloxacin	Pigs (gram positive)	≤0.5	1	≥ 2
Enrofloxacin	Horses	≤0.25	0.5	≥ 0.5
Enrofloxacin	Cattle (BRD)	≤0.25	0.5–1	≥ 2
Pradofloxacin	Dogs, Cats	≤0.25	0.5–1	≥ 2
Danofloxacin	Cattle (BRD)	≤0.25	0.5	≥ 1

* The breakpoint listed for ciprofloxacin is based on human use, not veterinary use.

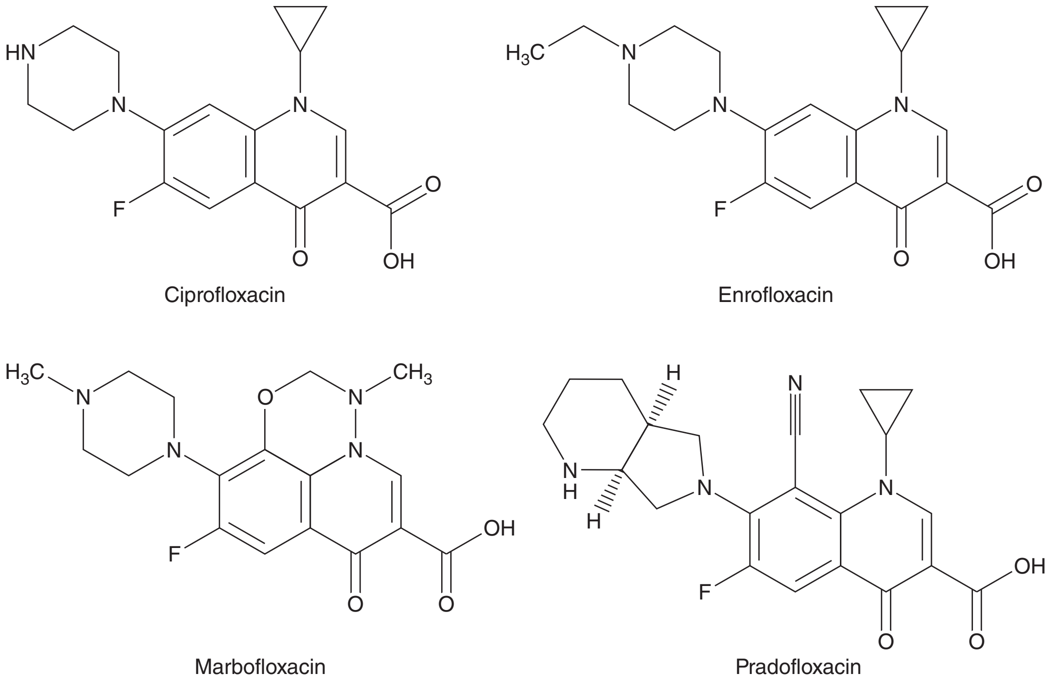

Figure 37.2 Structure of the fluoroquinolones. These representative structures show modifications from the basic structure presented in Figure 32.1. Features necessary for antibacterial activity are fluorine at position 6, ketone at position 4, and carboxyl at position 3. Addition of cyclopropyl, ethyl, or fluorophenyl at position 1 and of piperazine at position 7 increases the spectrum of antibacterial activity.

The *third-generation* fluoroquinolones have a bicyclic substitution at position 7, instead of a piperazine. This increased the activity to include a wider range of bacteria. In addition, a substitution at the 8 position on the ring enhances the bactericidal effects and improves the spectrum of activity to include more gram-positive and anaerobic bacteria. For example, the 8-methoxy substitution produces the drug moxifloxacin, a human quinolone with improved activity against gram-positive bacteria (Behra-Miellet et al., 2002; Aktaç et al., 2002; Pestova et al., 2000). Pradofloxacin, a veterinary quinolone with similar activity has a cyano substitution at position 8, which improves the activity against gram-positive and anaerobic activity compared to enrofloxacin and ciprofloxacin (Silley et al., 2007). This drug is discussed in more detail in Section Pradofloxacin Use in Dogs and Cats. A comparison of the activities of the veterinary fluoroquinolones against gram-positive and gram-negative bacteria can be illustrated in Figure 37.3 (using data from Wetzstein, 2005).

Mechanism of Action

Quinolones are bactericidal by inhibiting bacterial DNA replication and transcription. Two-stranded DNA is tightly coiled in the cell and must be separated for transcription and translation. To facilitate coiling, winding, and unwinding, the enzyme DNA gyrase allows the strands to be cut and reconnected. This allows coiling because negative supercoils can be introduced. DNA gyrase, a topoisomerase, consists of A and B subunits. The most common target site for quinolones is the A subunit of DNA gyrase coded by the gene *gyrA*. Mammals are resistant to the killing effects of quinolone antimicrobials because topoisomerase II in mammalian cells is not inhibited until the drug concentration reaches 100–1000 μg/ml. Bacteria are inhibited by concentrations of 0.1–10 μg/ml or less. Concentrations achieved in animals and people are much lower as seen by the Clinical Laboratory Standards Institute (CLSI, 2015) breakpoints (Table 37.2). Another target is the topoisomerase IV enzyme composed of subunits *parC* and *parE*. This site of action is less important for gram-negative bacteria but is a target of fluoroquinolones in some gram-positive bacteria such as streptococci and staphylococci (Ferrero et al., 1995). Among the newer generation fluoroquinolones – for example the veterinary drug pradofloxacin, and the human drugs moxifloxacin and gatifloxacin – the primary target for gram-positive bacteria may be the DNA-gyrase rather than topoisomerase IV, or these drugs also may be dual inhibitors against both targets (Intorre et al., 2007). Therefore, older fluoroquinolones have high activity against DNA-gyrase in gram-negative bacteria, but less against the topoisomerase IV of gram-positive bacteria. But the broader activity of newer fluoroquinolones can be attributed to higher affinity for both the DNA-gyrase in gram-negative and gram-positive bacteria,

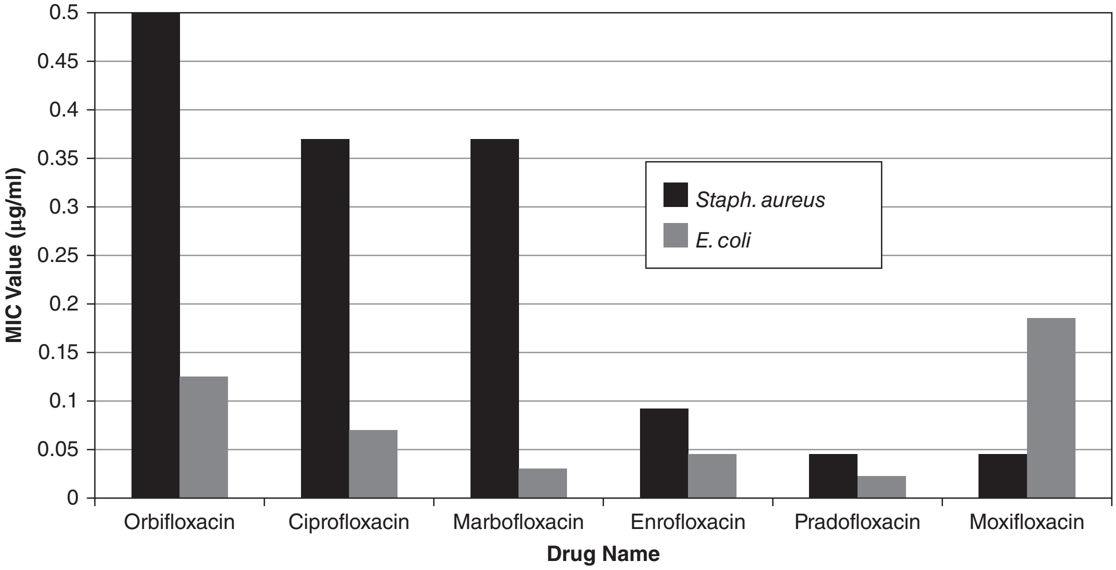

Figure 37.3 Comparison of fluoroquinolone MIC values for veterinary drugs, and compared to two human drugs, moxifloxacin and ciprofloxacin. Source: Data from Wetzstein, 2005.

as well as activity for the topoisomerase IV of gram-positive bacteria (Drlica and Zhao, 1997; Blondeau et al., 2004).

Antibacterial Spectrum

Fluoroquinolones in general exhibit good activity against most gram-negative bacteria, especially those of the Enterobacteriaceae. Representative minimum inhibitory concentration (MIC) values are shown in Table 37.3. *Escherichia coli, Klebsiella* spp., *Proteus* spp., *Salmonella* spp., and *Enterobacter* spp. are usually susceptible. *Pseudomonas aeruginosa* is variably susceptible and, when it is susceptible, usually has a higher MIC than other susceptible organisms. Against *P. aeruginosa,* ciprofloxacin is the most active (Rubin et al., 2008). Other bacteria susceptible to fluoroquinolones include intracellular organisms (*Rickettsia* spp., *Chlamydia,* and *Mycobacterium* spp.) and *Mycoplasma* spp.

Gram-positive bacteria are variably susceptible. *Staphylococcus aureus, Staphylococcus pseudintermedius,* and other *Staphylococcus* species usually are susceptible. However, the MIC values for staphylococci typically are higher than for gram-negative bacteria, and staphylococcal resistance to fluoroquinolones can be common.

In general, ciprofloxacin is more active than enrofloxacin against gram-negative bacilli, and enrofloxacin is more active against *Staphylococcus* species (Grobbel et al., 2007; Blondeau et al., 2012). Marbofloxacin activity usually falls between enrofloxacin and ciprofloxacin. The newer fluoroquinolones, such as moxifloxacin, gatifloxacin, and the veterinary drug pradofloxacin, have increased activity against gram-positive cocci and anaerobic bacteria (Behra-Miellet et al., 2002; Aktaç et al., 2002; Pestova et al., 2000; Silley, 2007) (Figure 37.3).

Factors that may affect activity are cations at the site of infection and low pH. Cations such as Al^{+3}, Mg^{+3}, Fe^{+2},

Table 37.3 Comparative microbiological data for common pathogens. Sources: Pirro et al., 1997, 1999; Asuquo and Piddock, 1993; Stegemann et al., 1996; Spreng et al., 1995; pradofloxacin FOI summary, and manufacturer data.

	MIC of bacteria (µg/ml)			
Drug	***Pasteurella multocida***	***Escherichia coli***	***Staphylococcus pseudintermedius***	***Pseudomonas aeruginosa***
Ciprofloxacin	0.015	0.03	0.25	0.5
Difloxacin	<0.05	0.11–0.23	0.25–0.91	0.92
Enrofloxacin	0.03	0.03–0.06	0.125	2.0
Marbofloxacin	0.04	0.125–0.25	0.23–0.25	0.94
Orbifloxacin	0.05	0.125–0.39	0.25–0.39	6.25–12.5
Pradofloxacin	0.008	0.03	0.06	

MIC values listed are MIC_{90} and represent an average from available published literature or manufacturer technical information.

and Ca^{+2} can bind a carboxyl group on the drug and significantly decrease activity. Low pH at the site of action also can affect the MIC (Ross and Riley, 1994), especially for drugs that have a piperazine at position 7 (Figures 37.1 and 37.2). For example, in urine, the MIC for fluoroquinolones may increase due to the presence of cations in the urine and low pH (Fernandes, 1988). This activity in urine may increase the MIC from fourfold to 64-fold. Fluoroquinolone activity in an abscess is not diminished despite the observation that in pus there is cellular material that can bind drugs, a low pH, and slow-growing bacteria (Bryant and Mazza, 1989). The presence of a foreign body, albumin, globulin, pus, anaerobic conditions, and dead bacteria did not affect the activity of fluoroquinolones (Rubinstein et al., 2000). The activity of fluoroquinolones in this milieu may explain its efficacy for treating infections associated with abscessation.

Susceptibility Testing

Susceptibility testing is performed either by the agar-disk-diffusion (ADD) method or broth dilutions (MIC test). The CLSI (CLSI, 2015) has published interpretive categories for breakpoints for most fluoroquinolones (Table 37.2). For isolates in the "intermediate" range, this is a category that implies that an infection due to the isolate may be appropriately treated in body sites where the drugs are physiologically concentrated or when a high dosage of drug can be used. Therefore, the dose can be increased in these instances, or successful treatment might be possible of the drug is applied topically or for a urinary tract infection. The MIC quality control ranges for wild-type organisms are also available from CLSI.

Resistance

There are multiple resistance mechanisms that have been identified for fluoroquinolones. These include: decreased drug permeability, increased drug efflux (efflux pumps), gyrase protecting enzymes, altered targets, and plasmid-mediated resistance. Of all the mechanisms listed, resistance most frequently develops via the *gyrA* mutation that codes for the A subunit of the DNA gyrase enzyme. A mutation at the serine-83 residue has been one of the most common, but at least 10 additional mutations at the *gyrA* gene have been identified to confer resistance (Ferrero et al., 1995). A mutation in the *parC* gene that codes for topoisomerase IV enzyme also is important (*grlA* mutation in staphylococci). A mutation in *parC* coding for resistant topoisomerase IV causes resistance when detected with mutations of *gyrA*, thus presence of both mutations usually produces high-level resistance. The multidrug resistance membrane efflux mechanisms reduce the accumulation of antibiotics in the bacteria. These efflux mechanisms are known to produce a high-level resistance to fluoroquinolones when they are present with other target site mutations (Zhanel et al., 2004). Because the efflux membrane pumps may affect other drugs, this may produce cross-resistance to other antimicrobials. Generally, there is cross-resistance among the fluoroquinolones. That is, although MIC values among susceptible strains can vary by a few dilutions, a fully resistant strain will typically be resistant to all the fluoroquinolones.

Resistance can occur through a multistep process (Everett et al., 1996). A single mutation can increase the MIC slightly (perhaps one dilution), and each subsequent mutation produces a progressively higher level resistance in a stepwise fashion. Unlike plasmid-mediated bacterial resistance, in which resistance may disappear after selective antibiotic pressure is removed, chromosomal (mutational) resistance exhibited by fluoroquinolone-resistant bacteria can be maintained in bacteria after drug administration is discontinued. Plasmid-mediated resistance has been found in *E. coli* and *Klebsiella* organisms, but the clinical significance of plasmid-mediated resistance has not been identified (Martinez-Martinez et al., 1998).

Clinical Resistance Problems

Resistance to fluoroquinolones has become a problem in human medicine and some investigators have attributed this to over-prescribing of these drugs. Resistance to fluoroquinolones by *E. coli, Staphylococcus aureus,* and *Streptococcus pneumoniae* has been well documented (Chen et al., 1999; Murphy et al., 1997; Sanders et al., 1995; Neu, 1992; Peña et al., 1995; Perea et al., 1999; Everett et al., 1996). Clinical resistance in human hospitals among staphylococci appeared relatively quickly after introduction of ciprofloxacin (Neu, 1992; Sanders et al., 1995; Hedin and Hambreus, 1991).

Resistant bacteria also have been identified in practically all animal species. Resistance in small animals has been documented for *E. coli, P. aeruginosa, Enterobacter, Proteus,* and other gram-negative bacteria. Resistance among staphylococci isolated from small animals has also been documented, particularly with methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (Couto et al., 2014; Bemis et al., 2009; Jones et al., 2007; Cole et al., 1998). The acquisition of specific *gryA/grlA* genes associated with phenotypic resistance to fluoroquinolones is strongly correlated with multidrug resistant methicillin-resistant *Staphylococcus pseudintermedius* (MRSP) (McCarthy et al., 2015).

Pseudomonas organisms have been particularly troublesome because single-step mutations are common for this bacteria, which can easily progress to high-level resistance. This is an important problem in companion animal medicine because, except for the fluoroquinolones, there are no other oral drugs with which to treat infections caused by *Pseudomonas aeruginosa.* Factors leading to resistant *P. aeruginosa* are an inadequate dosage and extended treatment at low doses. Various studies have confirmed increased frequency of resistance among *Pseudomonas aeruginosa* isolated from chronic infections in dogs (Petersen et al., 2002; Martin Barrasa et al., 2000; Cole et al., 1998).

Human Health Risks from Drug Resistant Bacteria

Infectious disease experts have warned that administration of fluoroquinolones to animals may be a public health concern. Transfer of fluoroquinolone resistance from animals to people has been suggested to occur for *Campylobacter* species (Endtz et al., 1991) and *Salmonella typhimurium* type DT-104 (Threlfall et al., 1995, 1997; Griggs et al., 1994). An increase in the incidence of resistant *Campylobacter jejuni* infecting people was linked to consumption of *Campylobacter*-contaminated chicken. The increased resistance occurred primarily after 1995, which coincides with the time that fluoroquinolones were approved for use in poultry as an additive to drinking water (Smith et al., 1999). Investigators have also associated resistance in salmonellae with veterinary use of fluoroquinolones in livestock (Piddock et al., 1998). However, resistant strains of *Salmonella typhimurium* may have occurred spontaneously because some of the resistant salmonellae have come from farms in which fluoroquinolones were not administered to animals (Griggs et al., 1994). Nevertheless, some scientists have warned that use of fluoroquinolones in livestock is a public health risk because it can potentially lead to resistant mutants of salmonellae being passed on to humans through the food chain. Because of these concerns, there have been limited approvals of fluoroquinolones for food-producing animals, and the extralabel use of fluoroquinolones is prohibited in food-producing animals in the United States. Because of the risk of *Campylobacter* resistance, all fluoroquinolone formulations for poultry have been removed in the USA.

In order to provide continued monitoring of fluoroquinolone resistance bacteria from food animals, the National Antimicrobial Resistance Monitoring System (NARMS) performs routine surveillance of antibiotic resistance data from human clinical samples, slaughter samples and retail meat samples. In their most recent report (available at: http://www.cdc.gov/narms/), NARMS concluded that “Overall, ciprofloxacin resistance has been consistently low among *Salmonella* isolated from all sources. Similarly, ciprofloxacin resistance in *E. coli* was absent or very low (0–1.7%) in isolates from retail meats, chicken and cecal samples.” They also found that for *Campylobacter jejuni* in retail chicken samples, resistance to ciprofloxacin was at its lowest level to date (11%), while ciprofloxacin resistance in *C. jejuni* isolated from chicken slaughter samples has not declined (22% in 2013).

Pharmacokinetics

Extensive pharmacokinetic data is provided in Table 37.4. These are presented as examples because there are far more pharmacokinetic studies available in the literature than can be presented in this table. A literature search of pharmacokinetic studies in animals will reveal many available studies in the entire array of species treated by veterinarians – ranging from large animal species of elephants and whales, to mice and small invertebrate species.

Mammals are relatively consistent in elimination half-life and volume of distribution. Reptiles with lower renal clearance generally demonstrate longer half-lives – as long as 55 and 36 hours for enrofloxacin in alligators and monitor lizards, respectively (Papich, 1999). There may be an allometric relationship in pharmacokinetic parameters among mammals ranging in size from mice to cattle (Bregante et al., 1999; Cox et al., 2004). The allometric relationship was improved considerably when the pharmacokinetic parameters were corrected for the percentage of protein-unbound enrofloxacin in the plasma (Table 37.5).

Because most of these drugs are administered either once daily or as a one-time administration, the elimination half-lives shown in Table 37.4 may have little effect on clinical outcome. For example, it does not appear that differences in half-life can account for different clinical results for skin infection treatment in dogs because enrofloxacin, which has the shortest half-life, and marbofloxacin, which has a long half-life, have both been reported to be effective when administered once daily (Paradis et al., 1990; Carlotti et al., 1995; Carlotti, 1996; Gruet et al., 1997; Koch and Peters, 1996; Ihrke, 1996, 1998; Cester et al., 1996). In cattle and pigs, enrofloxacin is approved for either a one-time dose of 7.5–12.5 mg/kg, or 2.5–5 mg/kg once daily for three to five treatments. With either regimen, the outcome is the same. Likewise, danofloxacin is approved for use in cattle at a dose of 6 mg/kg administered twice, or a single dose of 8 mg/kg, with similar outcomes.

Although the volume of distribution varies among animals (and among studies), these differences have not translated into differences in clinical efficacy. Marbofloxacin and orbifloxacin, which have a volume of distribution in the range of 1–2– l/kg, achieve effective skin concentrations and appear as clinically effective as drugs

Table 37.4 Pharmacokinetic comparison of fluoroquinolones

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (C_{max}) (μg/ml)	AUC[a] (μg · hr/ml)	%*F*	Assay[b]	Reference
Dogs									
Enrofloxacin	5	5	4.61	ND	1.75	11.65	Nd	HPLC	Frazier et al., 2000
Enrofloxacin	5	5	2.23	3.64	1.24	4.46	63.22	HPLC	Bidgood and Papich, 2005
Enrofloxacin	5.0 IV	5.0	2.7–3	5.0–5.6	—	4.05–4.34	—	HPLC	Intorre et al., 1995
Enrofloxacin	5.0	5.0	2.52	2.5	1.12 (oral)	7.27	72.3	HPLC	Cester et al., 1996
Enrofloxacin	5.8	5.0	4.4 (IV) 2.7 (oral)	4.5	1.44 (oral)	8.2	83.0	HPLC	Monlouis et al., 1997
Enrofloxacin	5.5	2.75–11.0	4.0 (oral)	nd	2.45 (oral)	16.32	Nd	Bioassay	Walker et al., 1992
Enrofloxacin	5.0	5.0	2.4	4.5	1.16 (oral)	3.9	100	HPLC	Küng et al., 1993a
Enrofloxacin	5.0	5.0–20.0[c]	4.8	4.2	1.6	8.15	—	HPLC	Stegemann et al., 1996
Ciprofloxacin	5.0	nd	3.17	2.23	0.35	4.18	43.0	HPLC	Cester et al., 1996
Ciprofloxacin	5.8[f]	nd	5.2	nd	0.34	7.2	Nd	HPLC	Monlouis et al., 1997
Ciprofloxacin	10.0	10.0–20.0	2.4	3.0	—	12.93	—	HPLC	Abadia et al., 1994
Ciprofloxacin	10.0	10.0–20.0	7.5	—	1.18 (oral)[d]	9.58 (oral)[d]	46[e]	Bioassay	Walker et al., 1990
Ciprofloxacin	25 (oral)	25–30 once daily	2.59	Na	4.44	22.5	58.36	HPLC	Papich, 2012
Ciprofloxacin	10 IV	15 IV once daily	3.72	2.39	Na	16.67	Na	HPLC	Papich, 2012
Difloxacin	5.0	5.0–10.0[c]	9.3	4.63	1.8 (oral)	12.93	96.0	nd	Manufacturer's data
Orbifloxacin	2.5	2.5–7.5[c]	5.6	1.5	2.33 (oral)	14.3	97–100	HPLC	Manufacturer's data
Marbofloxacin	5	5	7.63	1.54	3.63	42.08	104.6	HPLC	Bidgood and Papich, 2005
Marbofloxacin	2 SC	2 SC	13	Na	1.52	19.76	98.7	HPLC	Schneider et al., 1996
Marbofloxacin	4 SC	4 SC	13.4	Na	3.04	41.54		HPLC	Schneider et al., 1996
Marbofloxacin	4 oral	4 oral	12.5	Na	2.93	45.60	107	HPLC	Schneider et al., 1996
Marbofloxacin (2 studies)	2 IV	2 IV	12.4, 13.9	1.90, 2.25	Na	20.95, 18.60	Na	HPLC	Schneider et al., 1996
Marbofloxacin	2 oral	2 oral	14.0	Na	1.38	22.01	105	HPLC	Schneider et al., 1996
Marbofloxacin	2.0	2.0	9.8	1.4	1.35 (oral)	23.31	99.8	HPLC	Cester et al., 1996
Marbofloxacin	5.55 and 2.8 mg/kg	2.75–5.55[c]	9.5 (IV) 11 (oral)	1.27	2.0 at 2.8 mg/kg oral; 4.2 at 5.55 mg/kg oral	59.0	94.0	HPLC	Manufacturer's data
Marbofloxacin	5	5	4.07	Nd	1.41	8.74	Nd	Bioassay	Heinen, 2002
Marbofloxacin	2	2	9.07	Nd	1.47	13.07	Nd	Bioassay	Heinen, 2002
Marbofloxacin	2.75	2.75	10.9	Nd	2.53	35.44	Nd	HPLC	Frazier et al., 2000
Orbifloxacin	2.5	2.5	2.42	Nd	1.37	12.72	Nd	Bioassay	Heinen, 2002
Difloxacin	5	5	6.9	Nd	1.1	9.34	Nd	Bioassay	Heinen, 2002
Pradofloxacin	3	3–5	7.2–8	2.22	1.6	13.7	100	HPLC	Manufacturer's data

(*continued*)

Table 37.4 (*Continued*)

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*^{a} (hr)	Volume of Distribution^a (L/kg)	*Peak Concentration* (C_{max}) (µg/ml)	AUC^a (µg · hr/ml)	%*F*	Assay^b	Reference
Cats									
Levofloxacin	10	10	8.37	1.57	4.38	57.5	71.2	Bioassay	Albarellos et al., 2004
Enrofloxacin	5	5	6.7	2.5	1.0	20.3	107	HPLC	Seguin et al., 2004
Enrofloxacin (kitten)	5	5	3.7–6.3	1.8–3.9	0.5–1.1	5–12.7	33.7–72.8	HPLC	Sequin et al., 2004
Enrofloxacin	4.7	5^c	6.7	6.3	1.66 (oral)	7.2	100	HPLC	Richez et al., 1997b
Orbifloxacin	2.5	2.5–7.5^c	5.5	1.4	2.06	10.82	100	HPLC	Manufacturer's data
Ciprofloxacin	10 oral	10 mg/kg q12h oral	2.37	12.52	0.73	3.17	33	Bioassay	Albarellos et al., 2004
Ciprofloxacin	10 IV	10 mg/kg q12h	4.53	3.85	Na	17.20	Na	Bioassay	Albarellos et al., 2004
Pradofloxacin	5, 7.5 oral	5–7.5	7.3	4.45	2.1 at 5 mg/kg, 2.9 at 7.5 mg/kg	9.4 at 5 mg/kg; 13.3 at 7.5 mg/kg	69 tablets, 58% suspension	HPLC	Manufacturer's data
Horses									
Moxifloxacin	5.8	NR	34	5.3	3.12	50.9	Nd	HPLC	Gardner et al., 2004
Ciprofloxacin	5	NR	5.8	4.9 (Vdss)	0.6 (PO)	8.1	10.5	HPLC	Yamarik et al., 2010
Enrofloxacin	7.5	7.5 (oral)	5.9 (IV)	4.2	2.22	14.37	65.6	HPLC	Boeckh et al., 2001
Enrofloxacin	7.5	7.5 (oral)	5.3 (IV)	2.9	1.85	21.0	78.3	Bioassay	Haines et al., 2000
Enrofloxacin	5	5 (IV)	6.7	1.9	12.4 (IV)	25.3	Nd	HPLC	Papich et al., 2002
Enrofloxacin	2.5 and 5.0	5.0 (IV, IM) 5.0–7.5 (oral)	5.9–6.1	0.78 (5 mg/kg)	5.44	58.3	62.5	Bioassay	Giguère et al., 1996
Enrofloxacin (foals)	5.0	2.5–5.0	16.5	2.31	2.12 (10 mg/kg oral)	48.54	42.0	HPLC	Bermingham et al., 2000
Enrofloxacin	5	Nd	6.15 (IV)	2.32 (Vdss)	1.0 (approx)	13.8	55	HPLC	Peyrou et al., 2006
Marbofloxacin	2	Nd	7.42 (IV)	1.6 (Vdss)	0.8 (approx)	7.36	59	HPLC	Peyrou et al., 2006
Marbofloxacin	2	2	4.74 (IV)	1.17 (Vdss)	1.42 (IM)	11.27	87.9 (IM)	HPLC	Carretero et al., 2002
Marbofloxacin	2	2	7.56 (IV)	2.83	0.89 (PO)	8.26	62 (PO), 98 (IM)	HPLC	Bousquet-Melou et al., 2002
Ciprofloxacin	3.0	Not recommended	4.9 (IV) 10.7 (IM)	0.147	0.77 (IM)	6.97	96.0 (IM)	HPLC	Yun et al., 1994
Ciprofloxacin	5.0	Not recommended	2.6 (IV)	3.88	Na	4.83	6.8	Bioassay	Dowling et al., 1995
Ciprofloxacin	5 IV	Not recommended	5.80	4.93	Na	8.13	Na	HPLC	Yamarik et al., 2010
Ciprofloxacin	20 oral	Not recommended	3.63	38.075	0.63	3.30	10.47	HPLC	Yamarik et al., 2010
Orbifloxacin	2.5	2.5–5.0 (oral)	5.1	2.4	1.25 (oral)	9.06	68.3 (oral)	HPLC	Davis et al., 2006

Cattle									
Enrofloxacin	12.5	12.5	6.79	ND	0.96	14.95	ND	HPLC	Davis et al., 2007
Enrofloxacin	7.5	7.5	9.23	–	0.89	17.79	ND	HPLC	Foster et al., 2016b
Enrofloxacin	7.5	7.5	4.53	–	1.07	13.22	ND	HPLC	Foster et al., 2016b
Enrofloxacin	12.5	12.5	6.79	–	1.82	31.13	ND	HPLC	Foster et al., 2017)
Enrofloxacin	8	8	7.28	NS	0.81	7.51	ND	HPLC	TerHune et al., 2005
Enrofloxacin (1-day-old calves)	2.5	2.5–5 per day or 7.5–12.5 SC once	6.61	1.70	nd	13.94	nd	HPLC	Kaartinen et al., 1997
Enrofloxacin (1-week-old calves)	2.5	2.5–5 per day or 7.5–12.5 SC once	4.87	2.61	nd	6.73	nd	HPLC	Kaartinen et al., 1997
Enrofloxacin (lactating cows)	5.0	nd	1.68 (IV) 5.9 (IM)5.55 (SC)	1.63	0.73 (IM) 0.98 (SC)	7.42	82.0 (IM) 137.0 (SC)	HPLC	Kaartinen et al., 1995
Enrofloxacin (cows)	2.5	nd	2.82	2.98	nd	5.28	nd	HPLC	Bregante et al., 1999
Enrofloxacin (adult cattle)	5.0	2.5–5 per day or 7.5–12.5 SC once^c	2.3	1.65	0.73 (SC)	10.08	88.0 (SC)	HPLC	Richez et al., 1997a
Enrofloxacin (calves)	5.0	2.5–5 per day or 7.5–12.5 SC once^c	2.2	1.98	0.87 (SC)	7.99	97.0 (SC)	HPLC	Richez et al., 1997a
Enrofloxacin (beef steers)	5 IV	Na	5.15	1.59	Na	7.18	Na	HPLC	Idowu et al., 2010
Enrofloxacin (dairy cows)	5 IV	Na	3.69	1.56	Na	3.62	Na	HPLC	Idowu et al., 2010
Ciprofloxacin (calves)	2.8	nd	2.4	2.5	0.27	nd	53.0 (oral)	HPLC	Nouws et al., 1988
Danofloxacin	6	6	4.21	ND	1.7	9.72	ND	HPLC	TerHune et al., 2005
Danofloxacin	1.25	Nd	2.25	2.04	0.28	132.85	289	Bioassay	Shem-Tov et al., 1998
Danofloxacin	5	Nd	2.9	ND	0.82	4.7	78 (IM)	HPLC	Mann and Frame, 1992
Danofloxacin	1.25	Nd	6.26	4.0	0.18	2.9	91	HPLC	Apley and Upson, 1993
Marbofloxacin	10 IM	10 IM, once	17.5	Nd	7.92	52.66	Na	HPLC	Vallé et al., 2012
Marbofloxacin (Buffalo calves)	4 SC	4 SC x 3 days	7.37	Nd	1.63	14.17	Na	HPLC	Baroni et al., 2014
Goats									
Danofloxacin	1.25	Nd	4.67	3.8	0.33	2.29	110 (SC)	HPLC	Aliabadi and Lees, 2001
Enrofloxacin	5	5	1.1	1.3	6.7	6.7	ND	HPLC	Rao et al., 2000
Marbofloxacin	2	2	7.2	1.3 (Vdss)	1.9	8.44	100.7 (IM)	HPLC	Waxman et al., 2001

(*continued*)

Table 37.4 (*Continued*)

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (*C*$_{max}$) (μg/ml)	AUC[a] (μg · hr/ml)	%*F*	Assay[b]	Reference
Sheep									
Enrofloxacin	5	10	4.8	1.15	2.69	57.5	98.07	HPLC	Bermingham and Papich, 2002
Enrofloxacin	2.5	5.0 mg/kg/day SC	3.73	2.18	0.78 (IM)	5.47	85.0 (IM)	HPLC	Mengozzi et al., 1996
Enrofloxacin	2.5	5.0 mg/kg/day SC	3.8	1.3	0.6 (oral)	10.4	60.6 (oral)	Bioassay	Pozzin et al., 1997
Enrofloxacin	2.5	nd	2.5	1.53	nd	8.98	nd	HPLC	Bregante et al., 1999
Alpacas									
Enrofloxacin	5 and 10	5–10	13.0	1.6	7.8 (SC), 15.3 (PO)	58.4	90 (SC), 29 (PO)	HPLC	Gandolf et al., 2005
Elephants									
Enrofloxacin	2.5 (PO)	2.5 (PO)	18.4	Nd	1.31	34.93	Nd	HPLC	Sanchez et al., 2005
Mice									
Enrofloxacin	10.0	nd	1.48	10.5	nd	2.45	na	HPLC	Bregante et al., 1999
Rats									
Enrofloxacin	7.5	nd	1.8	4.78	nd	5.65	nc	HPLC	Bregante et al., 1999
Rabbits									
Enrofloxacin	7.5	nd	2.2	4.94	nd	5.52	nd	HPLC	Bregante et al., 1999
Enrofloxacin	7.5 (IV)	nd	1.87	3.97	na	5.38	na	HPLC	Aramayona et al., 1996
Enrofloxacin	5.0 (IV)	5.0	2.18 (IV)	4.4	na	3.89	na	HPLC	Cabanes et al., 1992
Enrofloxacin	5.0 (IM)	5.0	1.8	na	3.04	3.84	92.0	HPLC	Cabanes et al., 1992
Enrofloxacin	5.0 (IV)	5.0	2.5	2.12	na	8.6	na	HPLC	Broome et al., 1991
Enrofloxacin	5.0 (oral)	5.0	2.4	na	0.45	5.4	61.0	HPLC	Broome et al., 1991
Moxifloxacin	5	nd	1.84	2.12	1.3 (PO)	6.28	75.5 (PO)	HPLC	Fernández-Varón et al., 2005
Marbofloxacin	2 (IM)	2	4.33	1.65	1.8	12.44	100	HPLC	Abo-El-Sooud and Goudah, 2010
Marbofloxacin	5 oral	5 oral	8	Na	1.73	10.5	Na	LC-MS	Carpenter et al., 2009
Chickens									
Ciprofloxacin	5.0	5.0–15.0 IM, SC, oral	9.01	2.02	4.67	78.04	70.0	Bioassay	Atta and Sharif, 1997
Enrofloxacin	10.0	10.0	5.6 (IV)	5.0	1.88 (oral)	16.17	89.2	HPLC	Knoll et al., 1999
Ciprofloxacin	5.0	5.0	9.0	2.0	4.67	78.04	70.0 (oral)	Bioassay	Atta and Sharif, 1997
Enrofloxacin	10.0	10.0	10.3 (IV)	4.3	2.44 (oral)	34.51	64.0	HPLC	Anadón et al., 1995
Enrofloxacin	10 (PO)	NR	14	Nd	1.5	35	Nd	HPLC	Da Silva et al., 2006
Camels									
Enrofloxacin	2.5	2.5 IM, SC	3.58	1.4	1.44 (IM)	18.95	85.0 (IM)	Bioassay	Gavrielli et al., 1995
Harbor Seals									
Marbofloxacin	5	5	4.96	1.29	3.44	30.91	Nd	HPLC	KuKanich et al., 2007

Invertebrates									
Enrofloxacin Cuttlefish	10	10	1.0	Nd	10.95	26.71	Nd	HPLC	Gore et al., 2005
Sea Urchin	10 inject	10 inject or 10 mg/L water bath	38.8	0.45	90.92	1,200	Na	HPLC	Phillips et al., 2016
Sea Stars	5 inject	5 inject or 5 mg/L water bath	42.76	0.713	18.9	345.85	Na	HPLC	Rosenberg et al., 2016
Pigs									
Enrofloxacin	2.5		345	3.34	1.17	5.97	150	HPLC	Zeng and Fung, 1997
Enrofloxacin	2.5 IM	2.5 IM	12.06	1.85	1.17	19.18	74.53	HPLC	Anadón et al., 1999
Enrofloxacin	2.5 IV	Nd	9.64	1.41	Na	25.69	Na	HPLC	Anadón et al., 1999
Enrofloxacin	2.5 IM	2.5 IM	9.3	3.22	0.695	8.903	Na	HPLC	Bimazubute et al., 2010
Enrofloxacin	2.5		7.73	3.5	0.61 (IM)	9.94	95.0 (IM)	HPLC	Richez et al., 1997a
Enrofloxacin	2.5		5.5	3.95	0.75 (IM)	5.03	101 (IM)	Bioassay	Pijpers et al., 1997
Enrofloxacin	10.0 oral		nd	nd	nd	27.0	73.0–80.0	HPLC	Gyrd-Hansen and Nielsen, 1994
Enrofloxacin	7.5 SC	7.5 SC	26.6	6.4	1.10	47.86	Na	HPLC	Messenger et al., 2012
Ciprofloxacin	3.06	nd	2.57	3.83	0.17	2.88	37.0	HPLC	Nouws et al., 1988
Danofloxacin	5	Nd	8.0	ND	0.8	6.0	76 (IM)	HPLC	Mann and Frame, 1992
Marbofloxacin (12 weeks)	8 IV	8 IM or IV	13.5	Nd	Nd	88.6	Na	HPLC	Schneider et al., 2014
Marbofloxacin (12 weeks)	8 IM	8 IM or IV	13.2	1.58	5.55	79.9	89.6	HPLC	Schneider et al., 2014
Marbofloxacin (16 weeks)	8 IV	8 IM or IV	18	Nd	Nd	102	Na	HPLC	Schneider et al., 2014
Marbofloxacin (16 weeks)	8 IM	8 IM or IV	16.7	1.75	5.86	106	105	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	8 IV	8 IM or IV	18.4	1.58	Nd	127	Nd	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	4 IM	8 IM or IV	15.4	Nd	3.38	56.9	90.0	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	8 IM	8 IM or IV	15.1	Nd	6.3	115	91.5	HPLC	Schneider et al., 2014
Marbofloxacin (27 weeks)	16 IM	8 IM or IV	15.2	Nd	15.5	228	90.1	HPLC	Schneider et al., 2014
Fish									
Enrofloxacin trout	5.0 and 10.0	5.0 mg/kg q24h	24.0 and 30.0	3.22 and 2.56	0.945 and 1.28	109.2 and 171.3	42 and 49	Bioassay	Bowser et al., 1992
Enrofloxacin red pacu	5.0	5.0 mg/kg q48h IM	29.0	nd	1.64 (IM) 0.8 (oral)	46.3 (IM)	57.0 (relative)	HPLC	Lewbart et al., 1997
Enrofloxacin Atlantic salmon	10.0	5.0 mg/kg q24h oral	131.0	22.4	0.29 (oral) 0.54 (oral)	84.3 1.3 (IP)	89.0 (IP) 46 (oral)	Bioassay 66 (IM)	Stoffregen et al., 1997

(*continued*)

Table 37.4 (*Continued*)

Drug	Dose studied (mg/kg)	Recommended daily dose (mg/kg)	*Half-life*[a] (hr)	Volume of Distribution[a] (L/kg)	*Peak Concentration* (C_{max}) (µg/ml)	AUC[a] (µg · hr/ml)	%*F*	Assay[b]	Reference
Enrofloxacin Korean Catfish	10 IV	10	17.4	3.93	Na	60.34	Na	LC/MS	Kim et al., 2006
Enrofloxacin Korean Catfish	10 oral	10 oral	34.13	Na	1.02	38.43	64.6	LC/MS	Kim et al., 2006
Turtles									
Enrofloxacin Yellow-bellied sliders	5 IM	5 IM	17.6	Na	6.28	75	Na	HPLC	James et al., 2003
Enrofloxacin Yellow-bellied sliders	10 oral	10 oral	32.8	Na	3.44	53.2	93.6	HPLC	James et al., 2003
Enrofloxacin, Red-eared sliders	10 IP	10 IP	47.6	2.5	10.36	308.8	Na	HPLC	Giorgi et al., 2013
Enrofloxacin Loggerhead sea turtles	10 oral	Na	37.8	2.17	4.1	261	Na	Bioassay	Jacobson et al., 2005
Enrofloxacin Loggerhead sea turtles	20 oral	20 (once per week)	54.4	0.93	21.3	1799	Na	Bioassay	Jacobson et al., 2005
Frogs									
Enrofloxacin African Clawed Frog	10 IM	10 IM	5.32	0.84	10.85	57.59	Na	HPLC	Howard et al., 2010
Enrofloxacin African Clawed Frog	10 SC	10 SC	4.08	0.92	9.79	47.42	Na	HPLC	Howard et al., 2010

Na = Data not available or not applicable.
Nd = Not determined, NR = not recommended.
$t_{1/2}$ = Half-life of the terminal portion of the plasma concentration vs. time curve.
AUC = Area under the curve of the plasma concentration vs. time curve.
C_{max} = Maximum plasma concentration after administration of oral or IM dose.
%*F* = Percentage of oral or IM administered dose absorbed (determined from comparison of IV dose).

[a] Half-life, volume of distribution, and AUC are from an IV dose unless otherwise noted. Apparent volume of distribution is typically from the area method. Where the dose was administered by a non-IV route, the Vd reported is Vd/F.

[b] Assay type = Assay using HPLC is able to distinguish between enrofloxacin and ciprofloxacin, and values shown in table represent enrofloxacin. Assays performed by bioassay represent the parent drug and active metabolites. Bioassay may include concentrations of ciprofloxacin.

[c] Registered dose with the FDA in the United States. In some European countries doses may vary or may not include the flexible range. In most cases, when treating non-*Pseudomonas* infections, the lowest dose in the range listed is used.

[d] After multiple dosing with ciprofloxacin, the C_{max} was 1.18 mg/ml and the 12 hr AUC was 9.58 mg/hr/ml.

[e] Oral absorption of ciprofloxacin estimated from a comparison of independent oral and IV studies.

[f] These parameters determined after administration of 5.8 mg/kg of enrofloxacin.

Note that in enrofloxacin studies, ciprofloxacin also was detected at varying concentrations (depending on species and study). Ciprofloxacin pharmacokinetics were measured in those studies but is not reported in this table. The ciprofloxacin data can be found in the studies referenced.

Table 37.5 Plasma/serum protein binding of fluoroquinolones in animals (% bound)

Animal	Enrofloxacin	Ciprofloxacin	Marbofloxacin	Orbifloxacin	Pradofloxacin
Camel	17–24				
Cattle	56, 36–45, 60, 46	31, 70, 19, 49.6, 33.8			
Sheep	69				
Horse	22	37		21 (at 1 μg/ml)	
Pig	27	23.6, 35			
Dog	15–25, 27, 35, 72	44, 18.5	9.1, 22	13.24 (at 1 μg/ml)	30–35 28.8–31
Rabbit	53, 50, 35, 6.0	33, 28			
Chicken	21	30			
Mouse	42				
Rat	50				

Sources: Villa et al., 1997; Gavrielli et al., 1995; Nouws et al., 1988; Aramayona et al., 1994; Idowu et al., 2010; Davis et al., 2007; Bidgood and Papich, 2005, and author Papich's unpublished data.
When two or more values are listed, they represent results from independent studies.

such as enrofloxacin with a volume of distribution of 2.5–5 l/kg. Heinen (2002) demonstrated that even though approved doses may vary among enrofloxacin, marbofloxacin, and orbifloxacin in dogs, they are all capable of achieving pharmacokinetic–pharmacodynamic (PK-PD) targets (these targets are discussed in Section Pharmacokinetics / Pharmacodynamics). The data published (Bidgood and Papich, 2005, Walker et al., 1990, 1992; Messenger et al., 2012; Davis et al., 2007) or available from manufacturer technical information show that the fluoroquinolones, regardless of their volume of distribution and lipophilicity, achieve effective concentrations in tissues. Some exceptions are tissues not easily penetrated such as the central nervous system and eye.

Oral Absorption

Whether fluoroquinolones are administered with or without food has little effect on oral absorption. Feeding may delay the time to peak concentration (T_{max}), but this has little effect on clinical outcome because the extent of absorption, determined by the total AUC (area under the curve of the plasma concentration versus time curve) is not affected significantly. Administration with food has prolonged the terminal half-life when enrofloxacin was administered orally to reptiles (Papich, 1999), sheep, pigs (Gyrd-Hansen and Nielsen, 1994), and chickens (Anadón et al., 1995). This may be caused by a "flip-flop" pharmacokinetic effect introduced in Chapter 3 and discussed below in this section.

In clinical situations involving animals difficult to dose, fluoroquinolones may be added to a patient's food in order to provide a more convenient dosing form. For example, enrofloxacin tablets were placed in whole fish, which were fed to dolphins to produce good absorption (Linnehan et al., 1999), and enrofloxacin was injected into mice and fed to monitor lizards, also producing good absorption (Hungerford et al., 1997). Chewable tablets of enrofloxacin (Taste-Tabs) do not affect oral absorption (manufacturer's data).

In dogs, cats, and pigs, oral absorption of fluoroquinolones approaches 100% (Table 37.4), but in large animals, the extent of absorption has been less. Oral absorption of fluoroquinolones is variable in horses. Ciprofloxacin showed an oral absorption of only 6.8% in ponies (Dowling et al., 1995) and only 10% (mean) in adults (Yamarik et al., 2010). But enrofloxacin absorption is 63% (Giguère et al., 1996) in adult horses and 42% in foals (Bermingham et al., 2000). The value reported for adult horses is probably artificially high because the study used a bioassay that overestimates the concentration of enrofloxacin in plasma. With the exception of ciprofloxacin, other studies listed in Table 37.4 indicate relatively good oral absorption of these drugs in horses. Studies in ruminants produce conflicting results on oral absorption. In sheep, oral absorption was reported to be 61% (Pozzin et al., 1997), but absorption in ruminant calves was listed as less than 10% (Vancutsem et al., 1990). In sheep, absorption from oral administration was high (Bermingham et al., 2002). In addition, this study in sheep showed that when enrofloxacin was mixed with food and administered orally, absorption was high and half-life longer than after the IV dose. The long oral half-life was the result of slow release from the feed (possible adsorption) or slow emptying from the rumen ("flip-flop" effect). Absorption is also good in nondomestic ruminants such as the hoofstock kept in zoological parks (Gandolf et al., 2003; Gandolf, 2006). In camels (although not a true ruminant), oral absorption was reported to be negligible in one study (Gavrielli et al., 1995), but in another study absorption was 29% (mean) in alpacas and was capable of producing high enough concentrations to treat susceptible gram-negative pathogens at 10 mg/kg (Gandolf et al., 2005).

In birds, oral absorption of enrofloxacin is good, with effective levels being achieved by adding the drug to the bird's drinking water. This method of administration has been employed to treat sick pet birds (Flammer, 1998) and chickens (Knoll et al., 1999). However, as mentioned previously, administration to food-producing poultry is now prohibited in the United States. After continuous medication in the drinking water, steady-state plasma concentrations of enrofloxacin are 0.53 μg/ml (Knoll et al., 1999). In fish enrofloxacin absorption has been estimated to be 40–50% (Lewbart, 1998).

Intramuscular and Subcutaneous Injection

Absorption is virtually complete from intramuscular (IM) and subcutaneous (SC) injection. However, in some animals there was delayed absorption from IM or SC injections, which produced longer half-lives from these routes compared to IV administration (this is known as "flip-flop" absorption kinetics). Delayed absorption from injection is possibly due to a slow release from the injection site caused by tissue binding or tissue injury that disrupts blood flow. The large animal formulation of enrofloxacin (Baytril-100) is quite alkaline and can cause irritation at the injection site that can produce a delay in absorption.

In cattle, for example (Kaartinen et al., 1995), half-life was 1.68 hours from IV injection of enrofloxacin (5 mg/kg), but 5.9 and 5.55 hours from IM and SC injection, respectively, even though the extent of absorption was high. In the study by Davis et al. (2007) the half-life of enrofloxacin after a SC injection to calves at 12.5 mg/kg was 6.79 hours (mean), and 7.25 hours for the metabolite ciprofloxacin. For danofloxacin, the IV half-life in cattle was 0.9 hours and the IM half-life was 2.26 hours.

Metabolism

Metabolism of enrofloxacin to ciprofloxacin occurs via de-ethylation of the ethyl group on the piperazine ring. Activity against many bacteria is similar, but ciprofloxacin is somewhat more active than enrofloxacin against gram-negative bacilli, and enrofloxacin more active against *Staphylococcus* species (Riddle et al., 2000; Blondeau et al., 2012; Wetzstein, 2005; Grobbel et al., 2007). Together they produce an additive effect, and may broaden the spectrum compared to each drug alone. Other metabolites are produced from additional metabolism of ciprofloxacin, but these are minor and do not contribute to the antibacterial effects. There are also minor insignificant metabolites of some of the other drugs.

Examination of the extent of metabolism of enrofloxacin to ciprofloxacin in dogs and cats was reported in several studies (Küng et al., 1993a; Monlouis et al., 1997; Cester et al., 1996; Richez et al., 1997b; Kordick et al., 1997; Heinen, 1999; Seguin et al., 2004) in which high-pressure liquid chromatography (HPLC) was used to determine the specific concentrations of enrofloxacin and ciprofloxacin after enrofloxacin administration. The peak concentration (C_{max}) of ciprofloxacin accounts for approximately 20% in dogs and cats. Cattle produce more ciprofloxacin from enrofloxacin than most other species studied. The proportion of ciprofloxacin in plasma from administration of enrofloxacin to cattle has been measured as 25% (Richez et al., 1994), 31% (Foster et al., 2016a, 2016b), 37–39% (Idowu et al., 2010), and as high as 41% (Davis et al., 2007). The amount of ciprofloxacin produced in beef steers was slightly higher than dairy cows (ratio 64% and 59%, respectively) (Idowu et al., 2010). The studies by Davis et al. (2007), Foster et al. (2016a, 2016b), and Anadón et al. (1995) show that the ratio of ciprofloxacin: enrofloxacin concentrations in tissue can increase to greater than 1.0 after administration of enrofloxacin despite the low plasma concentrations of ciprofloxacin.

In pigs and foals there were only small traces of ciprofloxacin metabolized from enrofloxacin (Zeng and Fung, 1997; Bermingham et al., 2000; Richez et al., 1997a; Messenger et al., 2012). Older pigs may have greater metabolism to ciprofloxacin (Anadón et al., 1999).

In nonmammal species, the amount of ciprofloxacin produced from administration of enrofloxacin varies greatly. Studies in fish show that after administration of enrofloxacin, only 2% of the total fluoroquinolone concentration is comprised of ciprofloxacin (Lewbart et al., 1997). Reptiles and invertebrates tend to produce such small amounts of ciprofloxacin that it does not contribute to the plasma profile or AUC (Gore et al., 2005; Helmick et al., 1997; Howard et al., 2010; Hungerford et al., 1997; Papich, 1999; Raphael et al., 1994; Phillips et al., 2016; Rosenberg et al., 2016; Giorgi et al., 2013; James et al., 2003). In chickens, the concentrations of ciprofloxacin in plasma and tissues after administration of enrofloxacin are minimal (Knoll et al., 1999; Anadón et al., 1995).

If there are active metabolites produced, such as ciprofloxacin from enrofloxacin, this can cause errors in the interpretation of drug assays when a bioassay (microbiological assay) is used because a bioassay does not distinguish parent drug from active metabolite. Pharmacokinetic studies performed using bioassay techniques compared with studies using HPLC methods have demonstrated that bioassay can overestimate the combined enrofloxacin and ciprofloxacin concentrations in animals by as much as 70% for the AUC and 29% for the peak concentration (Cester et al., 1996). This finding agrees with another study in dogs: a microbiological assay overestimated the total enrofloxacin plus ciprofloxacin AUC determined by HPLC by as much as 30–70% (Küng et al., 1993b).

Excretion

The fluoroquinolones are primarily excreted via the kidneys by glomerular filtration and tubular excretion (Bregante et al., 1999). The role of tubular excretion has been demonstrated by showing that probenecid can decrease the clearance for some fluoroquinolones. For most of the drugs a major portion of the parent drug or metabolites can be recovered in the urine, with a smaller amount recovered in the feces. An exception is difloxacin (no longer marketed for animals), for which 80% of a dose was recovered in the feces and renal clearance accounted for less than 5% of the total systemic clearance.

Protein Binding

Protein binding is moderate for most of the fluoroquinolones (Table 37.5). The extent of protein binding has not limited distribution to tissues and has not produced a protein-binding interaction if displaced by another drug. Although protein binding is generally low, as shown in Table 37.5, there is a lack of consistency among studies, which is probably related to differences in technique used to measure protein binding. Protein binding can produce a reduction in antibacterial activity (Zeitlinger et al., 2008), but does not impair distribution to tissues. When in vivo ultrafiltration techniques were used in animals, the free drug concentration (protein unbound) in tissue fluid exceeded the fraction unbound in plasma (Davis et al., 2007; Bidgood and Papich, 2005; Foster et al., 2016a, 2016b; Messenger et al., 2012; Hauschild et al., 2013).

Tissue Distribution

Distribution to most tissues is listed for each drug in the manufacturer's package insert or Freedom of Information (FOI) summary. Specific studies for enrofloxacin have been conducted to show that it distributes to bone (Duval and Budsberg, 1995), prostate (Dorfman et al., 1995), and skin (DeManuelle et al., 1998). Specific studies using in vivo ultraviltration techniques show that distribution of the unbound drug (microbiologically active concentration) generally exceeds the value predicted by the unbound concentration in plasma (Davis et al., 2007; Bidgood and Papich, 2005; Foster et al., 2016a, 2016b; Messenger et al., 2012; Hauschild et al., 2013). Thus, extracellular tissue fluid concentration of the unbound fluoroquinolone will generally be in equilibrium with, or exceed, plasma drug concentrations.

In tissues in which the fluoroquinolones accumulate intracellularly, high tissue concentrations are reported because measurement of tissue concentrations is typically performed by homogenizing the tissue, which disrupts cells and releases intracellular concentrations. Tissue concentrations measured in this manner represent both intracellular and extracellular concentrations and can overestimate active drug concentrations. Some tissues, such as liver and kidney, may have fluoroquinolone concentrations several-fold higher than corresponding plasma concentrations.

Fluoroquinolones attain moderately high intracellular concentrations in macrophages and neutrophils. The cellular: extracellular (C : E) ratio is often greater than 4, but usually less than 10 compared to plasma concentrations (Pascual et al., 1990; Tulkens, 1990; Easmon and Crane, 1985; Drusano et al., 1998). The reason for intracellular concentration is unclear (Drusano et al., 1998; Van Bambeke et al., 2006). Fluoroquinolones are moderately lipophilic and also have a slower efflux from these cells. In humans, ciprofloxacin has an intracellular half-life of 6.7 hours in neutrophils versus 3.7 hours in serum (Easmon et al., 1986).

Concentrations in urine may be several times higher than plasma concentrations. Concentrations of enrofloxacin, marbofloxacin, and orbifloxacin in urine of dogs are listed by the manufacturer to be 43, 40, and 84.5 μg/ml, respectively. One exception to the high urine excretion is difloxacin (no longer marketed) for which less than 5% of the dose is cleared in the urine. Fluoroquinolones are among the few drugs that adequately penetrate the prostate gland in sufficient concentrations to treat prostatitis caused by infection. Enrofloxacin concentration (determined by bioassay) in the prostatic fluid and prostate tissue exceeded serum concentration at all times after administration (Dorfman et al., 1995). There were no differences in tissue concentrations when infected prostate was compared to healthy tissue. Concentrations of other fluoroquinolones in prostate tissue have been reported by the manufacturers to be 3.36, 5.6, and 1.35 μg/gram for difloxacin, marbofloxacin, and orbifloxacin, respectively.

Pharmacokinetics / Pharmacodynamics

Minimum inhibitory concentration values for bacteria are listed in Table 37.3 and breakpoints for susceptibility testing listed in Table 37.2. Even though there are differences in potency among the currently available fluoroquinolones, a pattern is apparent: *Pasteurella,* such as the strains found in skin wounds and cause of respiratory infections in livestock, are the most susceptible; wild-type strains of the gram-negative enteric bacilli (e.g., *E. coli* and *Klebsiella*) also have low MIC values. The gram-positive cocci such as *Staphylococcus aureus* and the common canine skin pathogen *Staphylococcus pseudintermedius* have MIC values at a somewhat higher range, and *P. aeruginosa* has MIC values that are among the highest for susceptible bacteria. Although not listed in Table 37.3, streptococci, enterococci, and anaerobic bacteria typically have MIC values high enough that they are usually in the resistant category. Fluoroquinolones of the third generation, such as pradofloxacin (Figure 37.3) have lower MIC values for gram-positive bacteria than the second-generation drugs.

Fluoroquinolones are bactericidal and they act in a concentration-dependent manner rather than a time-dependent manner. The exposure to the bacteria has been measured by using the maximum peak concentration (C_{max}) in relation to the bacteria MIC and expressed as the C_{max} : MIC ratio. A C_{max} : MIC ratio that is at least 8–10 times (i.e., a peak concentration that is 8–10 times the MIC) is desirable. A high C_{max} : MIC ratio is optimal to decrease emergence of resistance (Drusano et al., 1998; Blaser et al., 1987), but the area-under-the-curve (AUC) to MIC ratio (AUC : MIC) may be used to predict efficacy. When low C_{max} : MIC ratios were achieved, the mutant strains that occur spontaneously were not suppressed, and resistance emerged because these mutant strains have MIC values that are at least four to eight times that of the parent (wild-type) strain (Drusano et al., 1993).

The area under the curve (AUC) for a 24-hour dose interval in relation to the MIC is expressed as the AUC_{24} : MIC ratio. It is understood that the AUC represents the protein-unbound concentration. An AUC : MIC ratio of 125–250 has been associated with the optimum antibacterial effect (Lode et al., 1998; Hyatt et al., 1995; Dudley, 1991; Nicolau et al., 1995; Wright et al., 2000). According to USCAST (2015, http://www.uscast.org/) the free-drug AUC : MIC targets for a 2-$\log_{10}$ reduction (99% reduction) is 140, 65, 187, and 34 for Enterobacteriaceae, *Pseudomonas aeruginosa, Staphylococcus aureus*, and *Streptococcus*, respectively, based on experimental conditions.

These targeted C_{max} : MIC and AUC : MIC ratios were based on in vitro or in vivo studies performed with immunosuppressed laboratory animals or on clinical studies involving people with serious illness (Forrest et al., 1993; Blaser et al., 1987; Sullivan et al., 1993).

As reviewed by Wright et al., (2000), there is evidence that for some clinical situations, AUC : MIC ratios as low as 30–55 are necessary for a clinical cure, since the study in which 125 was cited involved critically ill human patients. The USCAST evaluation cited above (2015, http://www.uscast.org/) lists clinical AUC : MIC targets above 70 for Enterobacteriaceae and *Pseudomonas aeruginosa,* and 34 for *Streptococcus*. A lower AUC : MIC ratios for stereptococci than for gram-negative bacteria was also shown by Ambrose and Grasela (2000), in which they presented data and reviewed the relevant publications.

Some veterinary studies have shown that the ratios necessary for clinical results may not be as high as reported from studies in laboratory rodents or people (Lees and Aliabadi, 2002).Many veterinary patients treated with fluoroquinolones are not as immunosuppressed as laboratory animals or ill as people that participated in some clinical studies. Therefore the targeted ratios may be less in veterinary patients than cited above. For example, if one compares the AUC in Table 37.3 to representative MIC values from Table 37.4, lower AUC : MIC ratios in some patients appears adequate.

Dose Guidelines

Specific clinical uses are discussed in this chapter for the major species. Doses listed in Tables 37.4 and 37.6 cover a wide MIC range among susceptible bacteria, from as low as 0.03 μg/ml to as high as 1.0 μg/ml. The upper end of the dose is limited by safety (such as gastrointestinal or central nervous system effects); the lower dose is determined by efficacy. There is no advantage to frequent dosing (multiple times/day) as long as a sufficiently high C_{max} : MIC or the same AUC : MIC is achieved; therefore, the doses discussed for mammals and listed in Table 37.4 are intended for once-daily administration, or for some uses in cattle and pigs, a single high dose may be used for respiratory infections (Table 37.7). The single high dose regimen has not been tested for animals other than pigs and cattle.

Clinical Use

Dogs and Cats

The administration of fluoroquinolones to dogs and cats constitutes one of the largest applications of these drugs for veterinary medicine. They have been used for over 25 years for infections of the skin, soft tissue, oral cavity, urinary tract, prostate, ear, wounds, respiratory tract, and bone (Paradis et al., 1990; Ihrke and DeManuelle, 1999; Ihrke, 1996; Ihrke et al., 1999; Carlotti et al., 1999; Griffin, 1993; Hawkins et al., 1998; Dorfman et al., 1995; Cotard et al., 1995). The first veterinary quinolone was enrofloxacin, and veterinarians now have experience with marbofloxacin, orbifloxacin, and pradofloxacin (and occasionally others in different countries) (Table 37.1). The efficacy of the fluoroquinolones was established for indications listed on the labels approved by the US Food and Drug Administration (FDA), European authorities, and licensing in other countries.

At the approved doses, orbifloxacin, enrofloxacin, and marbofloxacin reach therapeutic targets in dogs against susceptible bacteria, even though the doses and pharmacokinetics vary among the drugs (Heinen, 2002). The efficacy of enrofloxacin and marbofloxacin has been

Table 37.6 Dose recommendations for enrofloxacin in exotic animals

Animal	Dose	Route	Interval	Reference
Alligator	5 mg/kg	IV, oral	Every 96 hr	Helmick et al., 1997
Savanna monitor	5 mg/kg (10 mg/kg for *Pseudomonas* spp.)	IM, oral	Every 96 hr	Hungerford et al., 1997
Burmese python	5 mg/kg (higher doses for *Pseudomonas* spp.)	IM	Every 48 hr	Young et al., 1997
Indian star tortoise	5 mg/kg	IM	Every 24 hr	Raphael et al., 1994
Red-eared slider	5 mg/kg	oral, IM	Every 72 hr (oral), every 48 hr (IM)	James et al., (unpublished data)
Gopher tortoise	5 mg/kg	IM	Every 24–48 hr	Prezant et al., 1994
Bottlenose dolphin	5 mg/kg	oral	Every 24 hr	Linnehan et al., 1999
Parrot and cockatoo	7.5–15 mg/kg	oral	Every 12 hr	Flammer, 1998
Fish (ornamental)	5 mg/kg	IM, oral, or IP	Every 48 hr	Lewbart, 1998

These recommendations are based on an analysis of pharmacokinetic data and limited clinical experience. There have been no well-controlled efficacy studies or safety studies in these animals.

demonstrated specifically for canine pyoderma, including deep pyoderma, through published reports (Ihrke and DeManuelle, 1999; Ihrke, 1996; Paradis et al., 1990; Carlotti et al., 1999; Ihrke et al., 1999; Koch and Peters, 1996). Although efficacy has been shown for treating pyoderma in animals, guidelines for treatment recommend that they not be used as a first-choice agent (designated as "second tier" or "second-line" antibiotics) (Beco et al., 2013; Hillier et al., 2014). Another common use of fluoroquinolones in small animals is for treatment of urinary tract infections (Weese et al., 2011). In some patients, a 3-day course of treatment is sufficient for a clinical cure (Westropp et al., 2012).

In addition to treatment of infections in these common sites, fluoroquinolones have been used to treat rickettsial infections (Breitschwerdt et al., 1990, 1999) and *Bartonella* infections in cats (Kordick et al., 1997). Against *Rickettsia rickettsii*, enrofloxacin is equally as effective as doxycycline or chloramphenicol (Breitschwerdt et al., 1990), but the success for eliminating *Bartonella* in cats has been equivocal (Kordick et al., 1997). Enrofloxacin has been used successfully to treat acute ehrlichiosis in dogs caused by *E. canis* and *E. platys* at a dosage of 5 mg/kg once daily for 15 days (Kontos and Athanasiou, 1998). However, success in treating chronic ehrlichiosis has not been demonstrated. Fluoroquinolones also have been used to treat infections caused by *Mycoplasma* and *Mycobacteria*. Although the activity against *Mycoplasma* can be variable (Hannan et al., 1997), it has been effective for some opportunistic mycobacterial infections in cats (Studdert and Hughes, 1992).

Ciprofloxacin Use in Dogs and Cats

Despite the availability of safe and effective veterinary-labeled fluoroquinolones (enrofloxacin, marbofloxacin, orbifloxacin), ciprofloxacin oral tablets, available in a generic formulation for people, are increasingly being used for treatment in dogs. Veterinarians in the USA can legally prescribe unapproved human-label drugs to non-food producing animals according to the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994. The oral absorption in dogs, according to published studies, is variable, inconsistent, and lower than in humans. Estimates from some studies (Abadia et al., 1994, 1995; Walker et al., 1990), indicate that oral absorption may approach 74 to 97%, but has been as low as 42%. In a more recent study (Papich, 2012) the average oral absorption was 58.4%, but with high variability (coefficient of variation, CV 45.4%). The range in oral absorption was from approximately 30% to 98%. The variable oral absorption appeared to be caused by the incomplete and inconsistent dissolution of the human generic oral tablet. A larger population pharmacokinetic study (Papich, 2017) showed a low and variable absorption with a C_{max} of 1.19 μg/ml, AUC of 13.8 μg h/ml, and half-life of 4.35 hours. Simulations using a dose of 25 mg/kg oral, once per day

Table 37.7 Single high dose for fluoroquinolones used in veterinary medicine

Drug	Brand Name(s)	Species	Dose
Enrofloxacin	Baytril, and generic	Cattle	7.5–12.5 mg/kg SC
Enrofloxacin	Baytril, and generic	Pigs	7.5 mg/kg, SC
Danofloxacin	Advocin	Cattle	8 mg/kg, SC

would reach therapeutic targets only for bacteria with MIC values of 0.06 μg/ml, or less, which is far less than the human breakpoint for susceptible bacteria (Table 37.2).

Studies with ciprofloxacin in cats (Albarellos et al., 2004) showed low oral absorption at 10 mg/kg (33%). The authors concluded that a dose of 10 mg/kg every 12 hours might be sufficient for susceptible gram-negative bacteria with low MIC values, but this dose would likely not meet therapeutic targets against other bacteria.

Pradofloxacin Use in Dogs and Cats

Pradofloxacin (Veraflox®) is one of the newest fluoroquinolones (sometimes referred to as a third-generation fluoroquinolone; see Section Structure–Activity Relationships for differences). Other drugs that meet this definition are the human drugs grepafloxacin, gatifloxacin, gemifloxacin, and moxifloxacin. Because the older, second-generation fluroroquinolones (e.g., enrofloxacin, marbofloxacin, orbifloxacin) have less activity against gram-positive cocci and anaerobic bacteria (Figure 37.3), prafofloxacin have improved activity against some organisms in veterinary medicine (Blondeau et al., 2004; Lees, 2013). Pradofloxacin MIC_{90} values are 0.06–0.125 μg/ml for *Staphylococcus* spp., 0.06 μg/ml for *E. coli*, and 0.015 μg/ml for *Pasteurella multocida*. Pradofloxacin has been evaluated in dogs and cats for skin, soft-tissue, oral, and urinary tract infections (deJong et al., 2004; Spindel et al., 2008; Hartmann et al., 2008b; Litster et al., 2007; Mueller and Stephan, 2007). Pharmacokinetic studies are also available (Hartmann et al., 2008a; Hauschild et al., 2013). Susceptibility data indicate that it is more active than other fluoroquinolones against bacterial isolates from dogs and cats (deJong et al., 2004; Silley et al., 2007; Stephan et al., 2007b) (Figure 37.3). Because it is active against two targets of fluoroquinolones (topoisomerase IV and DNA gyrase) development of resistant mutants may be less likely (Wetzstein, 2005; Stephan et al., 2007a).

At a dose of 3 mg/kg orally it was effective for treatment of urinary tract infections in dogs and at 5 mg/kg was effective for canine pyoderma (Mueller and Stephan, 2007). At a dose of 5 mg/kg in a 2.5% oral suspension it was effective for urinary tract infections in cats (Litster et al., 2007).

Pradofloxacin is approved in the USA for cats only, and in other countries for both dog s and cats. Pradofloxacin has been safe in cats with respect to ocular lesions (Messias et al., 2008). The European label and US label are slightly different. In Europe, pradofloxacin is indicated for the treatment of dogs and cats for infections caused by *Staphylococcus pseudintermedius*, superficial and deep pyoderma caused by susceptible strains of *S. pseudintermedius*, acute urinary tract infections caused by susceptible strains of *E. coli* and *S. pseudintermedius,* and adjunctive treatment to mechanical or surgical periodontal therapy in the treatment of infections of the gingiva and periodontal tissues caused by susceptible strains of anaerobic organisms (*Porphyromonas* spp. and *Prevotella* spp.) The registered dose is 3 mg/kg once daily for 7–35 days, depending on the indication. For cats, pradofloxacin 25 mg/ml oral suspension is indicated for the treatment of acute infections of the upper respiratory tract caused by *P. multocida, E. coli,* and *S. pseudintermedius* and for the treatment of wound infections and abscesses caused by *P. multocida* and *S. pseudintermedius* in cats. The US label is more limited and only applies to cats for treatment of skin infections (wounds and abscesses) in cats caused by susceptible strains of *P. multocida, Streptococcus canis, Staphylococcus aureus, Staphylococcus felis*, and *S. pseudintermedius*. The dose in cats according to US labeling is 7.5 mg/kg daily and 5 mg/kg once daily on European labels. Breakpoints are established for testing pathogens from dogs and cats (Table 37.2).

Small Mammals

Enrofloxacin and other fluoroquinolone antibiotics are used frequently in small mammals such as rabbits, mice, rats, and exotic species for skin and visceral infections (Göbel, 1999; Cabanes et al., 1992; Broome and Brooks, 1991). Fluoroquinolones are popular for treatment in small mammals because of potent activity against gram-negative pathogens affecting these animals and have good oral absorption. Oral tablets of fluoroquinolones have been administered directly or crushed to make a suspension that can be conveniently administered orally to the small mammals mixed with water, fruit, or some other palatable flavoring. When mixed in a compounded formulation with these vehicles, enrofloxacin was stable for 56 days (Petritz et al., 2013). Another study showed that marbofloxacin tablets could be crushed and mixed with flavored vehicles for rabbits and was stable for 14 days (Carpenter et al., 2009).

Small mammals such as rodents and rabbits are prone to gastrointestinal disturbances and enteritis caused by overgrowth of bacteria, especially *Clostridium* organisms after administration of β-lactam and macrolide antibiotics. Because fluoroquinolones are not active against the anaerobic bacteria that compete with *Clostridium* organisms, bacterial overgrowth of pathogenic opportunistic bacteria has not been a problem as it has with other drugs, such as penicillins or macrolides.

Of the available drugs, enrofloxacin has been the most extensively studied. The doses listed in textbooks and review articles for mice, gerbils, hamsters, rats, and guinea pigs are 2.5–5.0 mg/kg up to 10–20 mg/kg IM, SC, or orally administered twice daily. The pharmacokinetics has been reported (Table 37.4), and there is some experience with the drug's efficacy. In rabbits oral enrofloxacin has been effective for improving clinical signs associated with pasteurellosis. The recommended dose of enrofloxacin for rabbits is 5 mg/kg IM, SC, or oral. Although it does not completely eradicate the bacteria in pasteurellosis in rabbits, it is considered the drug of choice (Göbel, 1999; Broome and Brooks, 1991). Marbofloxacin and moxifloxacin also have been studied in rabbits and pharmacokinetics were favorable (Abo-El-Sooud and Goudah, 2010; Fernández-Varón et al., 2005; Carpenter et al., 2009). The recommended dose of marbofloxacin for rabbits is 5 mg/kg oral, once every 24 hours (Carpenter et al., 2009).

Reptiles

The use of fluoroquinolones in reptiles has become popular because of their activity, safety, and convenience of administration (Papich, 1999; Jacobson, 1999; Rosenthal, 1999). Enrofloxacin has been studied more than any other drug in this class in reptiles. It is active against gram-negative organisms often implicated in serious infection of reptiles, including *Salmonella* spp., *Aeromonas hydrophilia, Klebsiella* spp., and *P. aeruginosa,* and its pharmacokinetics has been summarized in a review (Papich, 1999). It shows remarkable differences among the reptiles, but generally the elimination is much longer than in mammals or birds, which allows long dose intervals – as long as every 96 hours in some species (Table 37.4). The elimination rate of drugs in reptiles varies with the animal's body temperature, because body temperature affects metabolic rate. When enrofloxacin is administered, there is variable metabolism to the active metabolite ciprofloxacin among the reptiles. Elimination half-life ranged from 55 hours in alligators to 5.1 hours in tortoises (Young et al., 1997; Raphael et al., 1994; Helmick et al., 1997; Hungerford et al., 1997; Prezant et al., 1994). Monitor lizards, and pythons had half-lives of 36 and 17.6 hours, respectively. Turtles have long half-lives ranging from 18 hours to more than 50 hours that allow for infrequent administration of once per day to once per week (Table 37.4). Analysis of pharmacokinetic data and appraisal of clinical experience (Jacobson, 1999; Papich, 1999) suggest a range of doses (Table 37.6), but safety and efficacy studies have not been performed.

Pharmacokinetic studies have shown good absorption of enrofloxacin from IM administration, and this route may prolong the half-life, probably because of delayed absorption from the injection site. Although some authors have suggested that oral administration should be avoided in reptiles because of unreliable absorption, absorption was good after oral administration to alligators, lizards, and turtles (Helmick et al., 1997; Hungerford et al., 1997; James et al., 2003). Because of slow gastrointestinal transit time, oral absorption may prolong the half-life ("flip-flop" effect).

Birds

The fluoroquinolones are an important group of antibiotics for pet birds and exotics kept in zoo collections, but they are prohibited in poultry in the United States. Administration is via drinking water, oral gavage, or by injection. Fluoroquinolones have the advantage of good activity against bacterial pathogens important to birds, including *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Staphylococcus* spp., and for treatment of *Chlamydophila psittaci* (formerly called *Chlamydia psittaci*). Resistance is possible for *E. coli* and *Pseudomonas* spp., however, and activity against gram-positive cocci (e.g., streptococci and enterococci) is low. Although there is in vitro susceptibility of *Chlamydophila* to fluoroquinolones, experience suggests that enrofloxacin can decrease clinical signs but not eliminate the infections (Flammer, 1998). Therefore, fluoroquinolones are not recommended for mass medication of pet birds, and doxycycline is still the choice for this indication (discussed in Chapter 34).

For pet birds, the dose is higher than for mammals because the clearance is faster and metabolic rate is higher. A comparison of enrofloxacin, danofloxacin, and marbofloxacin in quails (Haritova et al., 2013) showed half-lives of 2–4 hours, and variable oral absorption among drugs (data not shown in Table 37.4). Enrofloxacin had much lower oral absorption in these birds than the other drugs studied. The Haritova paper also included tables of pharmacokinetic parameters for other birds. Pharmacokinetic studies of enrofloxacin in birds indicate a dose of 15 mg/kg IM or orally every 12 hours (Flammer, 1998; Flammer et al., 1991). Enrofloxacin has been administered to ducks at a dose of 10 mg/kg every 24 hours IM or orally. Enrofloxacin added to drinking water at a concentration of 0.3–0.5 mg/ml has been used to treat susceptible bacteria (Flammer et al., 1990). Enrofloxacin was well absorbed by this route, and as long as the bird is drinking, effective plasma concentrations can be attained. One concern with the IM injection is that it can produce irritation at the site of injection, which is problematic because birds have a limited muscle mass into which one can inject.

Fish

Fluoroquinolones have been considered for treatment of infections in ornamental fish and for use in aquaculture. These drugs are active against important gram-negative bacterial pathogens of fish, and they appear to be well tolerated. Enrofloxacin has been administered orally to rainbow trout kept in water maintained at 10° and 15°C.

Although oral absorption is less than in mammals, it was sufficient to produce effective plasma concentrations (Bowser et al., 1992). MIC values for pathogens infecting fish range from 0.0064 to 0.032 µg/ml for the most sensitive organisms to 0.25–0.45 µg/ml for *Streptococcus* spp. Thus the dose of 5 mg/kg should produce effective plasma concentrations for most susceptible pathogens (Bowser et al., 1992). In Atlantic salmon, enrofloxacin administered at 10 mg/kg intraperitoneally, intramuscularly, and orally was well absorbed from these routes, with no advantage of one route over another, but it produced a wide range of half-lives and Vd. Oral absorption in salmon was 46%, but the authors concluded that at 5 mg/kg this route would be suitable for therapeutic treatment (Stoffregen et al., 1997). Tissue concentrations were high, with concentrations detected at 120 hours after dosing.

Enrofloxacin has also been studied in red pacu as a model for other ornamental fish (Lewbart et al., 1997). For treatment of bacterial infections in ornamental fish, Lewbart (1998) recommends enrofloxacin at a dose of 5 mg/kg. This can be administered IM, IP, or orally with a recommended interval of every 48 hours, but the IM route produces the most predictable plasma concentrations. The oral dose can be prepared as a mixture of 0.1% in fish food (10 mg per 10 g of food). Enrofloxacin also has been added to water and used as a bath for fish in which the drug is absorbed across the surface area of the gill to produce systemic levels. In this treatment 2.5–5.0 mg enrofloxacin per liter is used as a 5-hour treatment bath repeated every 24 hours (Lewbart et al., 1997). The resulting peak plasma concentration after such a treatment was 0.17 µg/ml. Studies of the stability of enrofloxacin in water at various degrees of salinity and pH showed enrofloxacin to be stable when added to a water bath (unpublished results from the laboratory of the author, MGP). However, the effect of the drug on nitrifying bacteria in the water should be considered.

Invertebrate marine species also have been examined. Studies in cuttlefish showed that clearance was surprisingly rapid, and doses of 5 mg/kg every 12 hours would be necessary to maintain effective concentrations (Gore et al., 2005). However, the same study indicated that systemic absorption from immersion of cuttlefish in an enrofloxacin medicated bath was possible. In sea urchins and sea stars population pharmacokinetic methods were used to examine injections and water bath treatments (Phillips et al., 2016; Rosenberg et al., 2016). In sea urchins and sea stars the half-life was long (39 hours, 43 hours, respectively), and concentrations were maintained above a level needed to treat susceptible bacteria. The medicated bath treatment produced lower concentrations, but may still be adequate for treating some susceptible bacteria.

Horses

In horses, there is no fluoroquinolone approved for use in the USA, but these drugs, especially enrofloxacin, are often used in horses to treat infections that may be resistant to other common equine antimicrobials. Several pharmacokinetic studies have generated data for these drugs in horses to guide dosing protocols. These data, as well as clinical experience, have shown that this class of drugs can be valuable for treating infections in horses. Their valuable properties include the following: (i) ability to administer by oral, IV, and IM routes, although only enrofloxacin is available in an injectable formulation in the United States; (ii) spectrum of activity that includes staphylococci and gram-negative bacilli such as *Klebsiella pneumoniae, E. coli,* and *Proteus* spp.; (iii) spectrum of activity that does *not* include anaerobic bacteria, therefore posing less risk of disrupting bacteria in the intestine than other oral antimicrobials; and (iv) good safety profile in adult horses. Despite these advantages, the oral absorption is less than other animals, and more variable. Therefore, the analysis of susceptibility testing breakpoints resulted in lower values for equine isolates compared to other animals (Table 37.2). These breakpoints are based on an oral dose of enrofloxacin of 7.5 mg/kg once daily.

The oral absorption of enrofloxacin in horses has generally been in the range of 50–70% (Table 37.4). Most bacteria that infect horses are susceptible, but resistance is expected for streptococci and anaerobes. Strains of *Pseudomonas aeruginosa* may be resistant or only moderately susceptible. *Rhodococcus equi* can be resistant, and success in treating *Rhodococcus* infections in horses with enrofloxacin has not been encouraging (see Chapter 36 for recommendations to treat *Rhodococcus* in horses). Based on the studies cited in this section, as well as clinical experience to date, an injectable dose of enrofloxacin at 2.5 to 5 mg/kg once daily or an oral dose of 7.5 to 10 mg/kg once daily is recommended (Giguère et al., 1996). The higher oral dose is used to accommodate the decreased systemic availability from an oral dose. For orbifloxacin (Orbax), an oral dose of 5 mg/kg once daily is recommended (Davis et al., 2006). These doses meet PK-PD criteria for susceptible bacteria discussed earlier in this chapter. For marbofloxacin (Zeniquin), IV doses of 2 mg/kg q 24 h may be adequate for treatment of most gram-negative infections caused by Enterobacteriaceae (Bousquet-Mélou et al., 2002; Peyrou et al., 2004; Carretero et al., 2002). However, this dose would not be adequate for many gram-positive bacteria, such as *Staphylococcus* spp., with MIC values of 0.25 µg/ml or higher. The injectable formulation is not available in the United States; therefore, oral tablets of marbofloxacin would be required for administration. Marbofloxacin has systemic availability of approximately 62% in horses.

(Bousquet-Mélou et al., 2002). Oral dosing at 2 mg/kg may be adequate for susceptible Enterobacteriaceae with MIC values less than 0.2 μg/ml, but not for other bacteria. Doses higher than 2 mg/kg have not been studied in horses. Ciprofloxacin is not recommended because the oral absorption was poor in ponies and adult horses (Dowling et al., 1995; Yamarik et al., 2010). Enteritis may occur in horses from ciprofloxacin owing to the poor absorption and disruption of intestinal bacteria (Yamarik et al., 2010).

The method of administration for horses has been to (i) crush up tablets used in small animals; (ii) administer the injectable solution (2.27% or 10%) IM (neck muscle) or IV; or (iii) administer the concentrated 10% solution orally (Baytril-100 cattle formulation). All these methods appear to produce adequate plasma concentrations, except for the administration of the concentrated 10% solution (Baytril-100) orally. This solution has produced inconsistent and incomplete absorption in horses, possibly because of its insolubility in solutions of low pH (Haines et al., 2000). In other studies, absorption of this solution was better when horses were fed (Boeckh et al., 2001). This solution also has been associated with oral mucosal lesions in horses. Some clinicians have produced more consistent oral absorption and reduced mucosal irritation when the 100 mg/ml solution was compounded into a gel (Epstein et al., 2004).

Moxifloxacin (Avelox) is a human drug of this group and has been used on a limited basis for treatment of infections in dogs and cats caused by bacteria that have been refractory to other drugs. When administered to horses, moxifloxacin had favorable pharmacokinetics that could make it suitable for oral use in horses (Gardner et al., 2004). However, oral administration also produced diarrhea in the experimental horses studied, and one of these horses tested positive for *Clostridium difficile* toxins A and B. The spectrum of activity of this drug may be broad enough to disrupt the normal flora.

Ruminants

In cattle and sheep, the pharmacokinetics of enrofloxacin and danofloxacin has been reported (Table 37.4) and doses have been derived from these studies, clinical trials, or the approvals in various countries. Enrofloxacin and danofloxacin are approved for use in cattle in the United States and some European countries. These fluoroquinolones have been highly active against important pathogens causing bovine respiratory disease (BRD) in cattle. The MIC_{90} values listed for *Histophilus somni, Mannheimia haemolytica,* and *P. multocida* are 0.03, 0.06, and 0.03 μg/ml, respectively. Extralabel use is not allowed in food-producing animals (see Chapter 52 and 55). For enrofloxacin, the dose ranges from a single SC dose of 7.5–12.5 mg/kg, or treatment for 3 days at 2.5–5.0 mg/kg, once daily, SC. The withdrawal time is 28 days. Comparisons of enrofloxacin pharmacokinetics between dairy cows and beef steers did not show significant differences in parameters between the groups (Idowu et al., 2010). It is not approved for lactating cattle or dairy calves, but disposition into milk of lactating cows has been studied. Enrofloxacin is highly excreted in milk. (See Section Administration to Nursing, Pregnant, or Young Animals.)

Danofloxacin (Advocin®) is also approved for treatment of BRD in cattle. Danofloxacin contains either 25 mg/ml or 80 mg/ml of danofloxacin as the mesylate salt, depending on the country in which it is approved. It has a broad spectrum of activity, similar to enrofloxacin, including bovine isolates of *Pasteurella, Mannheimia haemolytica*, *Haemophilus somnus*, and *Mycobacterium bovis.* The breakpoints are listed in Table 37.2. The US approved label dose allows either a dose of 6 mg/kg twice, 48 hours apart, or a single dose of 8 mg/kg SC. The European formulation of 2.5% also allows for the intramuscular or intravenous routes at a dosage rate of 1.25 mg/kg. At this dose, three treatments should be given at 24-hour intervals. However, the higher dose is preferred because it is more likely to hit PK-PD targets. (PK-PD properties are discussed in Section Section Pharmacokinetics / Pharmacodynamics.)

The danofloxacin manufacturer reported a half-life of 4.35, and volume of distribution of 3.6 l/kg at the label dose of 6 mg/kg. Some earlier work at lower doses (Giles et al., 1991) reported similar results (half-life 4 hours and volume of distribution of 2.76 l/kg). Lung tissue concentrations were higher than the corresponding serum concentration and persist above the MIC for 12–24 hours after injection (Giles et al., 1991; Apley and Upson, 1993). In a comparison between enrofloxacin and danofloxacin (TerHune et al., 2005), danofloxacin produced plasma concentrations that were 56 times the MIC of 0.03 μg/ml, which is the MIC reported for North American BRD isolates. Although it is not legal to use fluroquinolones in the USA in an off-label manner, studies have also shown danofloxacin to be effective for bacterial enteritis in calves and is registered for this use in other countries.

Marbofloxacin (Forcyl®) 160 mg/ml solution is approved in some countries for use in cattle, and has also been tested in buffalo calves (Baroni et al., 2014). The dose studied in cattle has been 2 mg/kg per day for 3–5 days, or a single injection of 10 mg/kg, injected IM in the neck muscle. Vallé et al. (2012) concluded that a single high dose was as effective, and may reduce resistance, compared to lower doses.

Pigs

Enrofloxacin is approved in the United States for use in pigs for treatment and control of swine respiratory disease (SRD) associated with *Actinobacillus pleuropneumoniae, Pasteurella multocida, Haemophilus parasuis*, and *Streptococcus suis*. Efficacy and microbiological data (MIC distribution) have been reported for these pathogens in pigs (Grobbel et al., 2007). The pharmacokinetics of fluoroquinolones in pigs are shown in Table 37.4 and breakpoints for testing shown in Table 37.2. Most of the pharmacokinetic studies in pigs (Nielsen and Gyrd-Hansen, 1997; Anadón et al., 1999; Wiuff et al., 2002; Bimazumute et al., 2009) showed that the half-life of enrofloxacin ranged from 9 to12 hours. These studies were performed at a low dose. At the dose most often used clinically, 7.5 mg/kg once SC, the pharmacokinetics and tissue distribution were reported by Messenger et al. (2012). The Messenger study showed that at the dose of 7.5 mg/kg, the free drug concentrations in tissue exceed the predicted unbound concentration and attained target levels for swine respiratory pathogens. In all but one study, the ciprofloxacin concentrations were low and difficult to quantify, accounting for less than 10% of the corresponding enrofloxacin concentrations, or were below the assay limit of quantification (LOQ). One study (Anadón et al., 1999) that used older pigs, was an outlier and reported ciprofloxacin concentrations that were 51.5% of the corresponding enrofloxacin concentrations.

Marbofloxacin (Forcyl®, Marbocyl®) 16%, 10%, or 2% solution for IV or IM injection is approved in some countries (not the USA) for treatment of swine respiratory disease and metritis–mastitis–agalactia syndrome in sows. The dose in pigs is 2 mg/kg once daily for 3–5 days, or 8 mg/kg administered once, IM. The absorption from IM injection in pigs is not affected by the dose or concentration of the formulation (Schneider et al., 2014).

Administration to Nursing, Pregnant, or Young Animals

Nursing Animals

Distribution also has been measured for milk in rabbits and cattle. Enrofloxacin is excreted rapidly in the milk after administration. In cattle after administration of enrofloxacin at 5 mg/kg, enrofloxacin concentrations in milk parallel the concentrations in serum, with a C_{max} of 1.3–2.5 μg/ml, but concentrations of the active metabolite ciprofloxacin exceed those of enrofloxacin (Kaartinen et al., 1995; Tyczkowska et al., 1994; Rantala et al., 2002). In another study, the concentrations of ciprofloxacin in milk of dairy cows exceed the plasma concentration by 45 times (Chiesa et al., 2013). Danofloxacin distribution into milk of cows also exceeded serum concentrations (Shem-Tov et al., 1998). In rabbits the milk-to-plasma ratios were 3.6 and 2.6 for enrofloxacin and ciprofloxacin, respectively, after administration of 7.5 mg/kg IV (Aramayona et al., 1996).

The high distribution of fluoroquinolones into milk is apparently caused by active transport by proteins in the udder. One of the transporters responsible is the breast cancer resistance protein (BCRP), an ATP-binding cassette transporter (Real et al., 2011). Protein binding is high in milk, which may also trap enrofloxacin and ciprofloxacin (Aramayona et al., 1996). Despite these concentrations of fluoroquinolones in milk, the activity of enrofloxacin in mastitic milk is decreased, possibly owing to lower pH, chelation with cations, or other factors in milk that inhibit fluoroquinolone activity (Kaartinen et al., 1995). Although an experimental study showed promising results for treating mastitis caused by *E. coli* (Rantala et al., 2002), clinical results have been more disappointing (Suojala et al., 2010). In the United States, this would be considered extralabel use and is prohibited.

When administering fluoroquinolones to nursing animals, the amount in the milk should be considered because fluoroquinolones may cause arthropathy in some species of young animals (discussed further in Section Safety). Disposition into milk was studied in two mares after administration, and it was shown that although both ciprofloxacin and enrofloxacin were present in milk at levels that were as high or higher than the mares' plasma concentrations, the total doses administered to the foals via suckling were small, and the plasma concentrations in the foals were negligible (author's observations). Likewise, when ciprofloxacin was administered to cows, the concentration in milk was high, and delivered an oral dose of 0.5 mg/kg to suckling calves (Chiesa et al., 2013). However, the drug did not accumulate in calves and concentrations in the calves were one-tenth to one-fifth the concentration in the cow. Young nursing animals may have decreased oral absorption caused by interference from calcium in milk (see information on kittens in Section Young Animals).

Pregnant Animals

When administering fluoroquinolones to pregnant animals, there will be some drug transfer across the placenta because these drugs are lipophilic and have low protein binding, and drug transfer is not limited by tissue barriers. Placental transfer has been specifically examined in rabbits, in which it was shown that the more lipophilic drug, enrofloxacin, crossed the placenta to a greater degree (80%) than ciprofloxacin (5% placental transfer), which is less lipophilic (Aramayona et al., 1994). Despite the rather high transfer of enrofloxacin across the placenta, there have been no reports of adverse effects when fluoroquinolones were administered to pregnant animals. Manufacturer studies have not shown any adverse effects on pregnancy or reproduction.

Young Animals

There is a risk that fluoroquinolones may cause damage to the developing cartilage of young animals. This is discussed more thoroughly in the Section Safety. There have been few pharmacokinetic comparisons of young animals versus older animals, but the studies available demonstrate that young animals were exposed to more drug than adults because of slower clearance. After administration of enrofloxacin, calves at 1 day of age had smaller volume of distirubion, longer half-life, and decreased clearance than at 1 week of age (Kaartinen et al., 1997). There also was a smaller amount of metabolism of enrofloxacin to ciprofloxacin in 1-week-old calves than in older ones. Rabbit pups exhibited lower clearance and longer half-life for enrofloxacin than adult rabbits (Aramayona et al., 1996). This pattern was also seen in horses: foals at 1–2 weeks of age showed little metabolism of enrofloxacin to ciprofloxacin after administration of IV and oral doses. Foals also exhibited slower clearance and longer half-life than adults (Bermingham et al., 2000).

In kittens, enrofloxacin was administered at 5 mg/kg via various routes (oral, IV, SC) (Seguin et al., 2004). There was no evidence of adverse effects or impaired excretion of enrofloxacin in kittens. The half-life in young cats (2, 6, and 8 weeks old) was shorter, and clearance more rapid, than in adult cats, Volume of distribution in 6 to 8-week-old cats was larger, and combined with the shorter half-life, produced lower plasma concentrations than in adults. Oral administration to kittens produced low plasma concentrations and it was hypothesized that interference with milk in nursing kittens may lower oral absorption (see Section Drug Interactions).

Safety

The fluoroquinolones have had a good safety record. For enrofloxacin, the LD_{50} in laboratory rats is 5000 mg/kg. When high doses were administered to animals during safety testing, one of the most common problems was gastrointestinal disturbances (nausea, vomiting, diarrhea), but these were usually produced at high doses and were not serious. Because most of these drugs used in animals do not alter the anaerobic flora of the gastrointestinal tract, there usually is minimal disruption of the intestinal bacterial population, even when these drugs are administered orally to rodents. There have been no reports of cutaneous drug reactions resulting from fluoroquinolone administration in the veterinary literature, but some of the freedom of information (FOI) summaries from manufacturers report an occasional reddening of the skin of dogs when high doses were administered.

There have been no reports of adverse effects on reproduction or pregnancy from administration of fluoroquinolones. Although the use in pregnant animals has been discouraged because of toxicity to developing cartilage, there have been no clinical reports where this effect has been described in offspring of treated animals.

After administration of high doses, adverse central nervous system (CNS) effects have been observed. The mechanism responsible for the CNS effects is probably through antagonism of the inhibitory neurotransmitter GABA. Fluoroquinolones injected rapidly IV or administered at high doses can induce CNS excitement. Fluoroquinolones can precipitate convulsions in some animals and these agents should be administgered cautiously to animals that are prone to seizures.

Safety issues have arisen in people that have been the subject of a warning by the FDA. On May 12, 2016, the FDA issued a warning to physicians advising that the serious side effects associated with fluoroquinolone antibacterial drugs generally outweigh the benefits for patients with sinusitis, bronchitis, and uncomplicated urinary tract infections who have other treatment options. For patients with these conditions, fluoroquinolones should be reserved for those who do not have alternative treatment options. During the FDA review, they revealed that fluoroquinolones when used systemically (i.e., tablets, capsules, and injectable) are associated with disabling and potentially permanent serious side effects. These side effects can involve the tendons, muscles, joints, nerves, and central nervous system. Some signs and symptoms of serious side effects include tendon, joint and muscle pain, a "pins and needles" tingling or pricking sensation, confusion, and hallucinations. Subsequently, the FDA is requiring sponsors to update their drug labels for all fluoroquinolone antibacterial drugs. The joint effects in young dogs and horses are discussed in Section Problems in Young Animals, but to our knowledge tendon injuries and the other concerns cited by the FDA for people have not been reported from the use of fluoroquinolones in animals.

Blindness in Cats

High doses of fluoroquinolones have caused ocular problems in cats from drug-induced changes in the retina (Corrado et al., 1987). This concern was precipitated by a report by Gelatt and colleagues (Gelatt et al., 2001) in which retinal degeneration was associated with enrofloxacin administration. This was followed by studies by the manufacturer in which toxicosis from enrofloxacin was described and new dose labeling was announced. The most common ophthalmological abnormalities were mydriasis, lack of menace reflex, and poor papillary light reflexes. Acute blindness may occur and retinal lesions were observed, which include increased tapetal reflectivity and attenuation of retinal blood vessels. In one review of safety studies (Corrado et al., 1987) nalidixic acid (one of the earlier quinolones) at 100 mg/kg/day, but not norfloxacin at 200 mg/kg/day produced both electrical and histopathological changes in feline retinas. In another review (Schluter, 1987), the author states that feline retinas are particularly sensitive to fluoroquinolones. When cats received 100 mg/kg of nalidixic acid there was suppression of electroretinographic waves and histological changes in the cones and rods, but ciprofloxacin treatment at the same dose to cats had no effect on the electroretinographic findings or on the fundus.

In studies performed by the manufacturer, enrofloxacin was administered to cats at doses of 0, 5, 20, and 50 mg/kg for 21 days (eight cats per group). There were no adverse effects observed in cats treated with 5 mg/kg/day of enrofloxacin. However, the administration of enrofloxacin at 20 mg/kg or greater caused salivation, vomiting, and depression. At doses of 20 mg/kg or greater, there were mild to severe fundic lesions on ophthalmological examination, including changes in the fundus and retinal degeneration. There was also abnormal electroretinograms, including blindness. Ford et al. (2007) reported on a study in 24 cats that received 3, 5, or 7 days of enrofloxacin at a high dose of 50 mg/kg (10 times the label dose). Ocular changes were observed by day 3 of the study. At this dose, enrofloxacin caused both retinal degeneration and systemic toxic effects. Because the blindness has been associated with high doses, the manufacturer has limited the dose to 5 mg/kg/day in cats.

The other fluoroquinolones approve for use in cats are orbifloxacin (Orbax), pradofloxacin (Veraflox), and marbofloxacin (Zeniquin). The current approved dose of orbifloxacin for cats is 2.5–7.5 mg/kg/day. In a published abstract (Kay-Mugford et al., 2001), orbifloxacin oral liquid was administered to cats at 0, 15, 45, and 75 mg/kg for at least 30 days (eight cats/group). This represents 6, 18, and 30 times the lowest label dosage. No ocular lesions were observed in any cats treated with 15 mg/kg. At the higher doses (18 and 30 times dose) there was tapetal hyperreflectivity in the area centralis and minimal photoreceptor degeneration. When marbofloxacin was administered to cats at 5.55, 16.7, and 28 mg/kg, representing 2, 6, and 10 times the lowest label dose, for 6 weeks, there were no ocular lesions in cats (manufacturer's data). At 55.5 mg/kg (10 times the lowest label dose) for 14 days there were also no lesions from marbofloxacin. As discussed above in the Section Pradofloxacin Use in Dogs and Cats, it has been administered safely to cats without producing ocular problems.

Problems in Young Animals

Fluoroquinolones can produce an arthropathy in young rapidly-growing animals (Gough et al., 1992; Burkhardt et al., 1997). The species most susceptible to developmental arthropathy are rats and dogs. Dogs between the ages of 4 and 28 weeks are the most susceptible. Affected dogs may show signs of lameness and joint swelling, but when the drug was discontinued, the lesions were reversible. Kittens, calves, and pigs are more resistant to this effect. For example, feeder calves and 23-day-old calves were administered 25 mg/kg for 15 days without evidence of articular cartilage lesions. Young foals are susceptible to the joint arthropathy from enrofloxacin at 10 mg/kg orally (Bermingham et al., 2000). However, studies in adult horses treated with enrofloxacin have not demonstrated articular toxicity (Bertone et al., 2000; Giguère et al., 1999).

The risk increases with higher doses and in most instances has been more clinically obvious only when the highest maximum dose was exceeded (e.g., at 25 mg/kg of enrofloxacin); however, even enrofloxacin dosages of 10 mg/kg/day have induced cartilage damage in young dogs. Lesions may occur in as few as 2 days after initiation of treatment (Yabe et al., 2001). The use of fluoroquinolones has been discouraged in children, but thousands of children have been treated with these drugs under a compassionate protocol with no reports of joint arthropathy.

Joint arthropathy is caused by toxicity to the chondrocyte that causes vesicles to form on the articular surface. The mechanism for damage to cartilage is via chelation of magnesium by the drug (Egerbacher et al., 2001). Magnesium is necessary for proper development of the cartilage matrix, especially in young, growing animals. Chelation of the magnesium results in a local magnesium deficiency leading to loss of proteoglycan in the articular cartilage. Studies in which magnesium was supplemented to decrease cartilage damage had equivocal results. (Magnesium added to the diet while oral drugs are administered would cause a chelation and significantly decrease oral absorption.)

Diseases or Conditions

There has been limited study of the disposition of fluoroquinolones in animals that have other conditions. In most of these instances, there were no changes in the drug's pharmacokinetics that would necessitate a change in dosage. Since the fluoroquinolones rely on both the kidneys and liver for clearance, insufficiency in one organ may result in compensation by the other clearance route. For example, renal failure may result in more reliance on hepatic clearance. In dogs with renal impairment, clearance of marbofloxacin was only slightly decreased and there was no significant effect on volume of distribution or mean residence time (Lefebvre et al., 1998). In camels deprived of water for 14 days and lost 12.5% of their body weight, there was little effect on the distribution, clearance, or half-life of enrofloxacin. However, water deprivation resulted in a slower and less complete absorption from a SC injection compared to normal camels or camels injected IM (Gavrielli et al., 1995).

Drug Interactions

Combinations with other antibiotics neither antagonize nor enhance the microbiological effects of fluoroquinolones. Fluoroquinolones will kill bacteria whether or not they are dividing (Lode et al., 1998). Therefore, use of a bacteriostatic agent should not interfere with the action of a fluoroquinolone. Although there is no evidence that other antibiotics produce a synergistic effect when administered with fluoroquinolones, they may broaden the spectrum of activity.

Fluoroquinolones are involved in some drug interactions, but few of these are serious. Drugs containing di- and trivalent cations (e.g., Ca^{2+}, Mg^{2+}, Al^{+3}, Fe^{+3}), such as antacids, sucralfate, and nutritional supplements, can inhibit oral absorption (Simon et al., 2010; Nix et al., 1989). The effect on oral absorption is caused by chelation of the di- and trivalent cations to the quinolone, which inhibits permeability through the intestine, and can also affect drug solubility in the intestine that is needed for absorption (Simon et al., 2010). Although these effects are well documented in people, especially for ciprofloxacin, the results have been inconsistent in animals. Sucralfate (containing Al^{+3}) did not decrease oral absorption of enrofloxacin in dogs, and ciprofloxacin absorption was so inconsistent that it was not possible to detect a statistically significant interaction (KuKanich et al., 2016).

Cations mixed with fluoroquinolones in extemporaneously compounded preparations, can chelate the drug and decrease oral absorption. Chelation occurs on the carboxyl group at the 3-carbon position (Figure 37.1). Fluoroquinolones may inhibit metabolism of some drugs through an interaction with hepatic metabolism. One such example is the inhibition of theophylline metabolism by enrofloxacin (Intorre et al., 1995), in which enrofloxacin significantly increased the peak concentration and decreased systemic clearance of theophylline in dogs. This interaction occurs because enrofloxacin inhibits the cytochrome P540 enzyme CYP 1A2 enzyme (Martinez et al., 2013). There are other substrates for this enzyme, but there have not been reports of studies to examine if other interactions are possible in animals.

Fluoroquinolone interactions with pharmacokinetics of some nonsteroidal antiinflammatory drugs (NSAIDs) have been observed in animals (Ogino et al., 2005; Sidhu et al., 2005). However the interaction is inconsistent. Enrofloxacin inhibited the clearance of flunixin, and vice versa (Ogino et al., 2005), but marbofloxacin increased the clearance of tolfenamic acid and decreased the concentrations (Sidhu et al., 2005). If a reaction with an NSAID is possible, it is likely because of competition for tubular secretion.

Formulations Available

Fluoroquinolones approved for small-animal use are available as oral preparations as tablets or chewable tablets. There are no oral liquid preparations currently available in the United States, but oral solutions are available in other countries (5 and 25 mg/ml). When oral liquid formulations have not been available, veterinarians have used compounding pharmacists to create oral liquid formulations from tablets dissolved in an aqueous vehicle (Petritz et al., 2013; Carpenter et al., 2009). There are two formulations of enrofloxacin, an IM injectable preparation for dogs (2.27% solution, 22.7 mg/ml), and an injectable for cattle (100 mg/ml). The solutions are not approved for IV administration, but veterinarians have occasionally administered this preparation IV. The IV administration has not produced serious problems, but one should avoid rapid IV injection; otherwise, CNS reactions are possible. In addition, mixing a solution with some intravenous solutions may cause chelation and precipitation in the IV line. Fluids of particular concern are those that contain calcium or magnesium. The formulation approved for SC injection in cattle in the United States is 100 mg/ml in an L-arginine base. This preparation may cause tissue irritation if injected in small animals. It is very alkaline and has produced oral mucosal lesions when used for oral administration to horses (Boeckh et al., 2001).

There is an otic preparation available (Baytril otic) containing 5 mg/ml enrofloxacin and 10 mg/ml silver sulfadiazine. Some veterinarians have also used topical administration of enrofloxacin for otitis externa caused by pseudomonads (Griffin, 1993, 1999; Rosychuk, 1994). Other otic preparations have been mixed extemporaneously by veterinarians, even though these are not approved or evaluated for efficacy. For example, veterinarians have mixed the 2.27% injectable solution of enrofloxacin with saline, water, or other topical ear solutions in a 1 : 1 to 4 : 1 ratio (e.g., 4 parts saline, 1 part enrofloxacin). Stability studies by the author (MGP) with HPLC analysis confirmed these solutions to be stable for 2 weeks at room temperature.

Danofloxacin mesylate (Advocin), available as 180 mg/ml (and in some countries 25 mg/ml), is used for administration to cattle, SC in the neck area. It has a 2-pyrrolidone and polyvinyl alcohol vehicle. Marbofloxacin (Zeniquin) is available in tablets for dogs and cats. There is an injectable formulation available in some countries for small animals, cattle, pigs, and horses (Marbocyl, Forcyl), but not in the United States. Orbifloxacin is available in tablet form for dogs and cats. There are many more formulations available around the world not listed here. For example, the European Medicines Agency web site (http://www.ema.europa.eu/ema/) lists 120 pages of fluoroquinolone formulations approved for animals in various countries. There is not enough space in this chapter to list all these formulations but a visit to the EMA web site, and the FDA approved drug web site (http://www.accessdata.fda.gov/scripts/animaldrugsatfda/).

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38

Antifungal and Antiviral Drugs

Jennifer L. Davis and Lara Maxwell

Antifungal Drugs

The need for safe and effective antifungal drugs has become important, particularly in small-animal medicine, with the recognition of serious systemic fungal diseases as well as the need for effective drugs to treat skin infections caused by dermatophytes and yeasts. Some animals are at a greater risk of fungal infections because they have other diseases or received medications that can produce immunosuppression, including hyperadrenocorticism, cancer chemotherapeutics, radiation therapy, or prolonged courses of corticosteroids. Fortunately, there have been many advances in the development of antifungal drugs in the last 20 years. Effective oral drugs are more widely used and there are newer, safer formulations of injectable agents. Figure 38.1 illustrates the sites of drug action for common antifungal drugs used in veterinary medicine. Unfortunately, there are only a few antifungal drugs that are approved for veterinary species (notable exceptions are topical products). Therefore, veterinarians often administer human-label drugs in an extralabel manner to animals.

Griseofulvin

Griseofulvin (Fulvicin U/F®, Fulvicin P/G®, Grifulvin V®, Grisactin®, Grisactin ultra®), is a fungistatic antibiotic produced by *Penicillium griseofulvin dierckx*. It is colorless, slightly bitter, and virtually insoluble in water. There are two types of preparations, the microsized and the ultramicrosized. Due to increased surface area, the ultramicrosized formulations have almost 100% bioavailability, whereas oral absorption of microsized formulations is lower and more variable (25–70%). The ultramicrosized preparations are not used often in veterinary medicine because of the higher cost. If the ultramicrosized form is used, the dose must be decreased to account for differences in absorption.

Mechanism of Action

Griseofulvin's selective toxicity is based on an energy-dependent uptake into susceptible fungi that occurs preferentially in fungal cells rather than mammalian cells. Once inside the cell, griseofulvin disrupts the mitotic spindle by interacting with polymerized microtubules, thus causing mitotic arrest in metaphase. Grossly this may appear as shortened fungal hyphae that have fewer branching points. This is known as the *curling* phenomenon. Griseofulvin may also interfere with cytoplasmic tubule formation, thereby inhibiting normal cellular trafficking.

Spectrum of Activity

Griseofulvin's activity is limited to organisms causing dermatophytosis, *Microsporum* spp., *Trichophyton* spp., and *Epidermophyton*. Fungal resistance to griseofulvin, caused by decreased energy-dependent uptake into the fungal cell, has not been reported to be a clinically important problem in veterinary medicine until recently. A 2013 study of feline and canine dermatophytosis showed therapy with griseofulvin failed to achieve both mycological and clinical cure in 16 dogs (39%) and four cats (40%), with some *M. gypseum* isolates from these animals reaching MIC values of >150 μg/ml (Nardoni et al., 2013).

Pharmacokinetics

Pharmacokinetic properties were reviewed by Hill et al. (1995). Griseofulvin distributes to the keratin of skin, hair, and nails and can be detected in the stratum corneum within hours of administration. Because of low water solubility, griseofulvin absorption is enhanced when given with a meal with high fat content. Only a small fraction of the dose is present in other body fluids or tissues. Absorption is nonlinear, and increases in doses may lead to a decreased fraction absorbed, as the rate-limiting step for absorption shifts from dissolution to solubility (Tanaka et al., 2013). The plasma half-life in the dog is 47 minutes (Harris and Riegelman, 1969);

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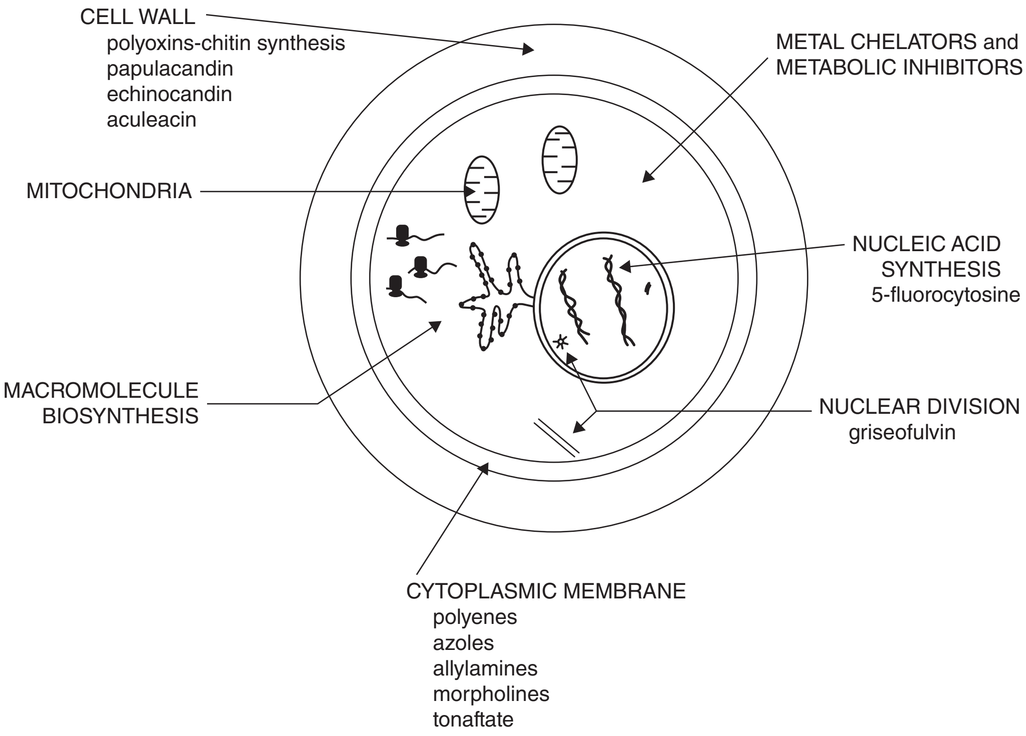


Figure 38.1 Schematic anatomy of a fungal cell and potential sites where antifungal drugs act.

however, the half-life at the site of action — the stratum corneum — is prolonged because the drug is bound tightly to keratinocytes and remains in the skin until these cells are shed. Thus, new hair or nail growth is first to become free of disease as keratin infected by fungus is replaced by new cells.

Griseofulvin is metabolized primarily by the liver to demethylgriseofulvin and the glucuronide. It is metabolized approximately six times faster in animals than in people, which is the reason animal doses are higher than human doses (half-life in dogs is less than 1 hour, compared to 20 hours in people) (Shah et al., 1972).

Clinical Use

Small animals: Griseofulvin is still used for treating dermatophytosis, but is being gradually replaced by azole drugs (discussed in Section Azole Antifungal Drugs). The recommended doses have varied, depending on the author. The label dose in dogs and cats for Fulvicin U/F® tablets is 11 to 22 mg/kg/day, but recommendations by specialists in dermatology have ranged from 44 mg/kg/day to 110–132 mg/kg/day in divided treatments (Scott, 1980). One review suggested a dose of 50 mg/kg once a day of the microsize formulation (Hill et al., 1995), and another review listed 25 mg/kg every 12 hours (deJaham et al., 2000), but the dose can be doubled for refractory cases. The most common dose is in the range of 50 mg/kg/day, which was confirmed in a report in which it was used in cats and was as effective as itraconazole for treatment of dermatophytosis (Moriello and DeBoer, 1995).

Griseofulvin is available in 125 and 250 mg capsules; 125, 250, and 500 mg tablets; and 125 mg/ml oral syrup. Often, at least 4 weeks are needed for successful therapy, and some patients require 3 months (or more) of continuous therapy. As long as 4 months may be necessary to treat infections of the nail bed (onychomycosis).

Large animals: Griseofulvin is approved at a dose of 2.5 g/day orally in adult horses for a minimum of 10 days. This translates to one packet of the powder formulation or one bolus per day, administered on feed. The dose for foals is half packet or half bolus per foal. Its use is limited to cases of dermatophytosis. There are currently no approved griseofulvin products for use in food animals in the United States. Nevertheless, when used off-label, griseofulvin has been effective in the prevention and treatment of dermatophytes in cattle (Reuss, 1978). Doses used are approximately 7.5–10 mg/kg for 7 to 35 days. At doses of 7.5 mg/kg orally once a day for 7 days, drug metabolites were still detectable in the liver of treated cattle at 10 days following the last administration (Tarbin and Fussell, 2013). A dose rate of 1 g/100 kg has been recommended for pigs for a duration of 30–40 days (Kielstein and Gottschalk, 1970). Because this drug is not approved, veterinarians must determine proper food animal withdrawal times for this drug prior to administration.

Adverse Effects

The most serious adverse effects associated with griseofulvin occur in cats and include leukopenia, anemia, increased hepatic enzyme activity, and neurotoxicosis (Helton et al., 1986). Ataxia in a kitten (Levy, 1991) and bone marrow hypoplasia in an 8-year-old cat (Rottman et al., 1991) have been reported. Prolonged treatment of eight cats with griseofulvin at the high end of the dosage range resulted in no untoward clinical, hematological, or hepatic side effects, suggesting that griseofulvin toxicity may be idiosyncratic (Kunkle and Meyer, 1987). Cats with feline immunodeficiency virus (FIV) appear to be at increased risk for griseofulvin-associated neutropenia (Shelton et al., 1990); however, toxicity has also been reported in FIV-negative cats (Rottman et al., 1991). The mechanism of this increased risk is unknown but may involve griseofulvin-enhanced binding of immune complexes to granulocytic cells in infected cats (Shelton et al., 1991).Griseofulvin should never be administered to pregnant cats. Its teratogenicity has been well-documented (Scott et al., 1975; Gruffydd-Jones and Wright, 1977). The teratogenic effects include cranial and skeletal malformations as well as ocular, intestinal, and cardiac problems (Scott et al., 1975). It has been given to pregnant horses with no apparent ill effect (Hiddleston, 1970); however, this may be dependent on the stage of pregnancy in which the drug was given. One report documented bilateral microphthalmia, brachygnathia superior, and palatocheiloschisis of the drug when given to a mare in the second month of pregnancy (Schutte and Van den Ingh, 1997). The labeled products for horses state not to administer to animals with impaired hepatic function.

Amphotericin B

Amphotericin B (Fungizone®, Abelcet®, Amphotec®, AmBisome®) is a polyene antibiotic with a large macrolide ring with a hydrophobic conjugated double-bond chain and a hydrophilic hydroxylated carbon chain and attached sugar (Figure 38.2) (Mechlinsk et al., 1970).

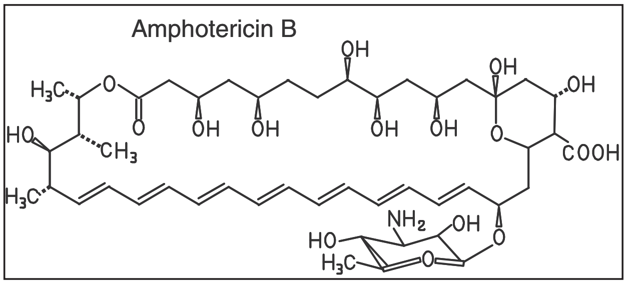


Figure 38.2 Amphotericin B.

It is a yellowish powder that is insoluble in water and somewhat unstable (Bennett, 1990). There are several formulations of amphotericin B available, including the conventional formulation, which is a micellar complex with the bile salt deoxycholate, and newer formulations that are lipid-based complexes. These are less toxic, but also more expensive (reviewed by Plotnick, 2000).

Amphotericin B lipid complex (Abelcet) is a suspension of amphotericin B complexed with two phospholipids. Amphotericin B cholesteryl sulfate complex (Amphotec, ABCD) is a colloidal dispersion of amphotericin B. The liposomal complex of amphotericin B (AmBisome) is a unilamellar liposomal formulation which, when reconstituted, produces small vesicles of encapsulated amphotericin B. Some investigators have attempted to achieve the benefits of lipid formulations without the added cost by mixing the deoxycholate salt in a 10 or 20% lipid solution (Intralipid). This formulation is stable for up to 3 weeks after mixing (Walker et al., 1998); however, the reports of the benefit of this emulsion versus the conventional formulation are inconsistent.

In comparison to the conventional formulation of amphotericin B, lipid formulations can be administered at higher doses to produce greater efficacy with less toxicity (Hiemenz and Walsh, 1996). Decreased toxicity is attributed to a selective transfer of the lipid complex amphotericin B, releasing the drug directly to the fungal cell membrane and sparing the mammalian cell membranes. Reduced drug concentrations in the kidneys and diminished release of inflammatory cytokines from amphotericin lipid complex compared to the conventional formulation may also prevent adverse reactions.

Mechanism of Action

The major action of amphotericin B is to bind ergosterol in the fungal plasma cell membrane, making the membrane more permeable and resulting in leakage of cell electrolytes and cell death (Brajtburg et al., 1990). At high concentrations, amphotericin B is thought to cause oxidative damage to the fungal cell (Warnock, 1991) or disruption of fungal cell enzymes. The selective toxicity of amphotericin B is based on its decreased binding to the major cell membrane sterol of mammalian cells (cholesterol) as compared to that of fungal cells (ergosterol).

Amphotericin B demonstrates concentration-dependent fungicidal activity. There is also a postfungal effect whereby an antifungal effect persists after drug concentrations have declined. This property allows for intermittent therapy (e.g., every other day in dogs).

Spectrum of Activity

The growth of strains of most veterinary fungal pathogens is inhibited in vitro at amphotericin B concentrations between 0.05 and 1.0 μg/ml and there is good correlation between the MIC values and clinical response (O'Day et al., 1987). Because of concentration-dependent killing, peak (C_{max}) concentrations should be two to four times above the MIC (C_{max}/MIC ratio of 2–4 : 1) (Goodwin and Drew, 2008).

Susceptible fungi include *Histoplasma capsulatum, Cryptococcus neoformans, Coccidioides immitis, Blastomyces dermatitidis, Candida* spp., and various species of *Aspergillus*. Amphotericin B has been indicated for treatment of mucormycosis, sporotrichosis, and phycomycosis (Drouhet and Dupont, 1987). Most strains of *Pseudallescheria boydii,* as well as some agents causing chromoblastomycosis and phaeohyphomycosis, are resistant to amphotericin. Clinical fungal resistance to amphotericin B, either primary or acquired, does not appear to occur commonly, although resistant strains occur in vitro. In most cases, these resistant strains contain decreased levels of membrane ergosterol (Pierce et al., 1978) and increased catalase levels may allow these fungi to be resistant to oxidative damage (Sokol-Anderson et al., 1988). The MIC concentrations were increased in some human patient populations, such as neutropenic patients (Dick et al., 1980), transplant patients (Powderly et al., 1988), and patients undergoing cytotoxic therapy.

The spectrum of activity also includes the protozoa *Leishmania* and is often included in protocols to treat human and canine leishmaniasis. The treatment of protozoa is discussed in Chapter 42.

Pharmacokinetics

Despite its long history of use, much is still unknown concerning the pharmacokinetics of amphotericin B, especially in veterinary medicine. It is poorly absorbed from the gastrointestinal (GI) tract and therefore must be given intravenously, locally, or intrathecally. Amphotericin B binds extensively (~95%) to serum proteins, mainly β-lipoprotein (Bennett, 1977). Much of the drug is thought to leave the vascular space and bind to cholesterol-containing membranes. The highest concentrations are found in liver, spleen, kidney, and lungs, with little accumulation in either muscle or adipose tissue. Concentrations of amphotericin B in fluids from inflamed pleura, peritoneum, synovium, and aqueous humor are about two-thirds of those in serum. Amphotericin B readily crosses the human placenta. Penetration into normal or inflamed meninges, vitreous humor, and normal amniotic fluid is poor. This differential distribution may explain treatment failures for infections in some tissues. Although amphotericin B binds ergosterol with higher affinity than cholesterol, it was suggested that because there are more binding sites for cholesterol in the body than for ergosterol, amphotericin B may be sequestered from its site of action (Bennett, 1977).

Clinical Use

Amphotericin B is used to treat a variety of fungal diseases caused by susceptible fungi, as listed above. Numerous dosage protocols for amphotericin B have been described in the veterinary literature. These are summarized in Table 38.1. One such protocol for small animals was published by Rubin (1986) that is still used today. During infusion, it should be mixed with 5% dextrose solution because it will precipitate if added to an electrolyte containing solution (e.g., lactated Ringer's solution). A solution of amphotericin B with 0.45% saline and 2.5% dextrose has been used successfully subcutaneously without any visible precipitation (Malik et al., 1996).

Amphotericin B is used only sporadically as a systemic antifungal in equine medicine and there are no pharmacokinetic data available on amphotericin in the horse. It is more often used as a local treatment in the eye, limbs, and upper airway. In cases of ocular fungal disease that do not respond to typical therapy, amphotericin B (0.2 ml of a 5 mg/ml solution) injected subconjunctivally q 48 h for up to three treatments can be used. This should provide a higher level of drug to the eye; however, it may produce localized toxic effects, including conjunctivitis and conjunctival necrosis. Intravenous regional limb perfusion (IRLP) has also been reported to successfully treat pythiosis of the lower limbs at a dose of 50 mg for one to two treatments (Dória et al., 2012). Side effects include limb edema with pain on palpation, and inflammation of the injection site; however, these signs are considered manageable and resolve after 14 days. Topical and intralesional therapy with amphotericin B has also been reported as a successful treatment for nasal conidiobolomycosis in the horse (French et al., 1985; Zamos et al., 1996). There are no reports of use of this drug in food animals, and there are no approved formulations for use in these species.

Combination therapy using amphotericin B and flucytosine has been shown to be synergistic against cryptococcal infections (see Section Flucytosine). Combinations of amphotericin B and azole antifungals have been less successful. Azole-induced depletion of fungal cell membrane ergosterol results in fewer binding sites on which polyene antifungals can exert their effect. Antagonism and synergism between these two classes of antifungal agents have been reported experimentally (Polak et al., 1982; Dupont and Drouhet, 1979). Because of the slower onset of action of azole antifungals, many clinicians recommend initial therapy of serious systemic fungal infections with amphotericin B, followed by longer, follow-up treatment with an azole therapeutic protocol.

Adverse Effects

The most important clinical toxicosis associated with amphotericin B therapy is nephrotoxicity. It is this effect

Table 38.1 Selected dosing protocols for amphotericin B in companion animals

Species	Formulation	Disease treated	Dosing protocol	Reference
Canine	Fungizone	Unspecified	Pretreatment with 0.9% sodium chloride followed by infusion of 0.5 mg/kg in 5% dextrose (D5W) over 4–6 hours IV q48h; a test dose of 0.25 mg/kg is sometimes recommended.	Rubin, 1986
Canine	Abelcet	Blastomycosis	Pretreatment with LRS at 2.5 times maintenance for 30 minutes followed by flushing the line with D5W and infusing 1 mg/kg amphotericin in D5W over 2.5 hours IV followed by LRS at 2.5 times maintenance for an additional 2 hours after treatment. Repeat q48h to a total cumulative dose of 8–12 mg/kg.	Krawiec et al., 1996
Canine	Abelcet	Unspecified	2–3 mg/kg IV 3 times per week diluted in 5% dextrose to a concentration of 1 mg/ml for a total of 9–12 treatments (cumulative dose of 24–27 mg/kg).	Grooters and Taboada, 2003
Canine	AmBisome	Leishmaniasis	3–3.3 mg/kg IV.	Oliva et al., 1995
Canine	Fungizone 40 ml sterile water and 10 ml 10% Intralipid	Leishmaniasis	Pretreatment with 50 ml/kg of 0.9% sodium chloride followed by 10 ml/kg 20% mannitol. Drug mixture infused over 30–60 minutes at incrementally increasing dosing from 1–2.5 mg/kg IV twice a week for a minimum of 8 injections.	Lamothe, 2001
Canine/feline	Fungizone in 0.45% saline with 2.5% dextrose	Cryptococcosis	0.5–0.8 mg/kg SC in 400 ml for cats or 500 ml in dogs given twice a week for a cumulative dose of 8–26 mg/kg.	Malik et al., 1996
Feline	Abelcet	Unspecified	1 mg/kg IV 3 times per week diluted in 5% dextrose to a concentration of 1 mg/ml for a total of 12 treatments (cumulative dose of 12 mg/kg).	Grooters and Taboada, 2003
Equine	Fungizone	Phycomycosis	0.38 gradually increased up to 1.47 mg/kg IV in 1 l 5% dextrose once daily.	McMullan et al., 1977
Equine	Fungizone	Pulmonary histoplasmosis	0.3–0.6 mg/kg IV in 1 l 5% dextrose once a day or every other day.	Cornick, 1990
Equine	Fungizone	Systemic candidiasis	0.1–0.5 mg/kg IV in 1 l 5% dextrose infused over 4–6 hours once daily.	Reilly and Palmer, 1994
Equine	Fungizone	Candida arthritis	0.33–0.89 mg/kg IV in 1 l 5% dextrose once a day or every other day.	Madison et al., 1995
Equine	Fungizone	Cryptococcal pneumonia	0.5 mg/kg IV in 1 l 5% dextrose as a 1-hour infusion once a day.	Begg et al., 2004
Avian	Fungizone	Aspergillosis	1.5 mg/kg q8–12h reconstituted in sterile water and then diluted 1 : 50 with 5% dextrose for 3–7 days.	Tully, 2000

on kidneys that is the most common reason for discontinuing therapy with amphotericin B. It is a dose-related, predictable toxic effect that occurs in almost every animal treated with the conventional formulation. Direct tubular damage occurs because amphotericin B binds to cholesterol in the tubular cells, which results in electrolyte leakage from the cells (primarily K^+ loss) and renal tubular acidosis (Bennett, 1990). Induced renal vasoconstriction and impaired acid excretion may also contribute to amphotericin B's renal toxicity (Greene, 1990). Renal vasoconstriction may be caused by induced increases in the eicosanoid synthesis in renal blood vessels. The tubular damage, along with the renal vasoconstriction, leads to both an acute and a chronic cumulative renal toxicosis. Clinically, the signs of kidney injury are seen as increases in creatinine and blood urea nitrogen (BUN). Electrolyte loading, fluid diuresis, and slow infusion of amphotericin B have all been shown to decrease the severity and the rate of development of renal toxicity. Therefore, common protocols for administration of amphotericin B to animals include pretreatment with sodium chloride IV solution (Rubin, 1986) with or without mannitol (Legendre et al., 1984), and a slow infusion. Slower infusion times are associated with less kidney injury (Rubin, 1986). If the dose administered during a single infusion exceeds 1 mg/kg, acute renal injury is likely (Butler and Hill, 1964).

Careful clinical monitoring will help decrease the risk of permanent renal injury. Urine sediment evaluation has been suggested to detect kidney injury earlier than serum biochemical alterations (Greene, 1990); thus, urine should be evaluated for proteinuria, cylinduria, and hematuria, as well as specific gravity. In addition, BUN, creatinine, and electrolyte concentrations should be monitored. Therapy should be temporarily discontinued when active urine sediment is detected or the serum creatinine increases. After stopping therapy, patients may undergo a fluid diuresis to decrease the azotemia. If BUN and creatinine return to near-normal reference values, treatment may be resumed. If azotemia does not improve, one should consider an alternative treatment.

Other adverse effects from amphotericin that are frequently observed in animals include phlebitis, fever, nausea, and vomiting. Measures to prevent the nausea and vomiting have included administration of antiemetic drugs such as chlorpromazine, maropitant, or metoclopramide prior to infusion (see Chapter 46). Hypokalemia, bronchospasm, and anemia/hemolysis have frequently been reported in humans and therefore should be monitored for in veterinary patients.

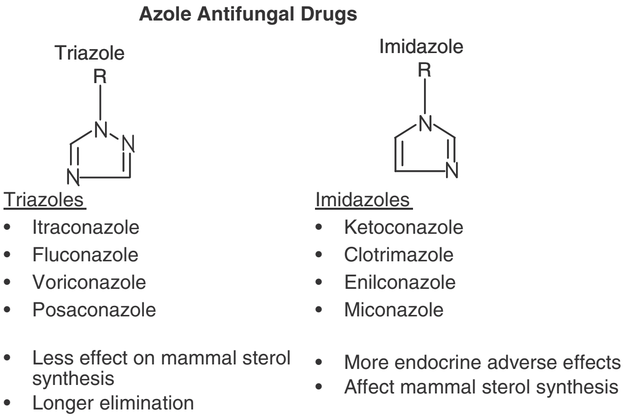


Figure 38.3 Azole antifungal drugs

Azole Antifungal Drugs

The azole antifungal drugs have a high safety profile, a broad spectrum of activity, and are available in topical, oral, and intravenous formulations. There are two main categories of azole antifungal drugs, the imidazoles (clotrimazole, miconazole, ketoconazole) and the triazoles (fluconazole, itraconazole, voriconazole) (Figure 38.3). Clotrimazole and miconazole are discussed in the section on topical therapy. The important physicochemical differences between azole antifungal drugs are summarized in Table 38.2.

Mechanism of Action

All azoles exert their antifungal effect on the cell membrane of fungi by inhibiting synthesis of the primary sterol of the fungal cell membrane, ergosterol. Inhibition of the P450–dependent lanosterol C_{14}-demethylase enzyme results in depletion of ergosterol and

Table 38.2 Comparison of the physicochemical properties and in vitro activity of commonly used azole antifungal drugs

					Activity		
Drug	**Solubility**	**pH Dependent**	**LogP**	**Protein binding**	**Yeasts**	**Aspergillus**	**Fusarium**
Ketoconazole	pi	Yes	3.78	>90%	+	±	−
Fluconazole	ss	No	0.54	10–12%	+	−	−
Itraconazole	pi	Yes	5.66	>98%	+	±	−
Voriconazole	vss	No	1.81	32–58%	+	+	±

pi, practically insoluble (<0.01 mg/ml); ss, slightly soluble (1–10 mg/ml); vss, very slightly soluble (0–1 mg/ml).

accumulation of C_{14}-methyl sterols in the cytoplasmic membrane of yeasts and filamentous fungi. This enzyme is also known as CYP51A or Erg11p and is encoded by the *ERG11* gene. Inhibition of this cytochrome P450 enzyme occurs via binding of the nitrogen (N_3 of imidazoles and N_4 of triazoles) to the heme iron atom of ferric cytochrome P450. This prevents the formation of the superoxide Fe^{+3} complex ($Fe^{+3}O^-$) needed for hydroxylation of methyl sterols. The result is an inability to demethylate C_{14}-methyl sterols and reduced synthesis of ergosterol. Sterols with less planar configurations are then incorporated into the fungal cell wall, which changes membrane fluidity and interferes with the barrier function of the membrane and with membrane-bound enzymes.

Azole drugs are generally fungistatic at concentrations achieved clinically, although there are exceptions for some fungal species, and for some drugs. The parameter that is best associated with clinical cure for azole drugs is the total exposure as measured by the area-under-the-curve in relation to the MIC (AUC/MIC ratio) (Goodwin and Drew, 2008).

The potency of each azole drug is related to its affinity for binding the P450 enzyme. The selective toxicity of each compound is directly dependent upon its specificity for binding fungal P450 more readily than mammalian P450. Imidazoles are less specific than triazoles and produce side effects in animals attributed to inhibition of P450 enzymes that are responsible for the synthesis of cortisol and reproductive steroid hormones. Azoles may decrease cholesterol, cortisol, androgen, and testosterone biosynthesis and may interfere with hepatic CYP450 enzymes that are important for drug metabolism and carcinogenic agents (Polak, 1990). These drugs also may inhibit the membrane transporter known as P-glycoprotein.

Interactions with Drug Metabolism

The inhibition of mammalian P450 enzymes is also responsible for drug–drug interactions that have been observed with the azole antifungals. When azoles are administered concurrently with other drugs that are metabolized by these enzymes, they can significantly increase the plasma concentrations of those drugs. Alternatively, when azoles are administered concurrently with drugs that induce the P450 enzymes, the concentrations of the azole drugs may be significantly decreased. The drug–drug interactions important to veterinary medicine are summarized in Table 38.3. The ability to inhibit mammalian P450 enzymes, and therefore the likelihood of drug–drug interactions, is greatest with ketoconazole (Aidasani et al., 2008) followed by itraconazole, voriconazole, and fluconazole.

Another method by which the azole antifungals can interfere with the absorption and pharmacokinetics of concurrently administered medications is through inhibition of the P-glycoprotein efflux pumps. These efflux pumps can be found in the intestine, where they limit the absorption of some substrates, as well as in the liver, kidney, eye, and CNS. At the intestinal level, there is a relationship between the P-glycoprotein efflux pump and the metabolism by intestinal CYP450 enzymes (Benet, 2009). Inhibition of both can have profound effects on systemic drug concentrations. Azole antifungals have the ability to inhibit P-glycoprotein pumps and therefore increase the oral absorption and tissue distribution of drugs within the body, particularly into protected sites, such as the blood–brain barrier and the blood–retinal barrier. The ability to inhibit P-glycoprotein is greatest with itraconazole, followed by ketoconazole and voriconazole (Wang et al., 2002). Fluconazole has little interaction with P-glycoprotein, which may explain why it has fewer significant drug interactions compared to the other azole antifungals (Yasuda et al., 2002; Wang et al., 2002).

Ketoconazole

Ketoconazole (Nizoral®), one of the imidazoles, became available in 1979. The results of successful use in veterinary medicine were published shortly thereafter (Legendre et al., 1982; Medleau et al., 1985). Ketoconazole is available in 200 mg tablets, and generic formulations are inexpensive and readily available.

Spectrum of Activity

Ketoconazole is most effective against yeast and dimorphic fungi such as *Candida*, *Malassezia pachydermatis*, *C. immitis*, *H. capsulatum*, and *B. dermatitidis*, as well as most dermatophytes with MIC values less than 0.5 μg/ml. It is less effective against *C. neoformans*, *S. schenckii*, and *Aspergillus* spp., with MIC values varying from 6 to >100 μg/ml (Hume and Kerkering, 1983).

Pharmacokinetics

Ketoconazole is relatively insoluble, except in an acid environment. It is not well absorbed orally unless there is acid secretion, such as after a meal. Ketoconazole is highly protein bound (>98%) and therefore does not penetrate into the cerebrospinal, seminal, or ocular fluid to a significant degree; although it does partition into milk. It distributes throughout the skin and subcutaneous tissue, making it effective for treatment of superficial and systemic fungal skin infections. The drug demonstrates nonlinear absorption and elimination kinetics, most probably due to saturation of solubility or metabolizing enzymes. It is biotransformed in the liver via O-dealkylation and aromatic hydroxylation and excreted mainly in the bile. Elimination half-life is approximately 2 hours in dogs.

Because ketoconazole is soluble only in acid aqueous environments (pH <3), gastric alkalizing agents (e.g.,

Table 38.3 Antifungal drug–drug interactions of significance in veterinary medicine[a]

	Drug/drug class	Result
Griseofulvin	Anticoagulants/coumarin or inandione derivatives	Griseofulvin is a hepatic enzyme inducer, which may increase the metabolism of these drugs, resulting in decreased anticoagulant effects.
	Barbiturates	Impaired absorption and therefore possibly impaired effectiveness of griseofulvin.
Amphotericin B	Bone marrow depressants	Increased risk of anemia or other blood dyscrasia.
	Corticosteroids	Exacerbation of hypokalemia, particularly with those drugs that have significant mineralocorticoid activity.
	Digoxin	Hypokalemia caused by AmpB increases the potential for digitalis toxicity.
	Neuromuscular blocking agents	Hypokalemia caused by AmpB enhances the blockade of nondepolarizing agents.
	Diuretics	Potassium depleting diuretics will exacerbate hypokalemia.
	Flucytosine	Synergism of AmpB with flucytosine may decrease the dose of AmpB necessary, therefore reducing the nephrotoxicity, however AmpB-induced renal dysfunction may increase 5-FC concentrations, thus increasing the potential for blood dyscrasias.
Azole Antifungals	Drugs that increase gastric pH	Decreases the absorption of those drugs with a pH-dependent solubility (ketoconazole and itraconazole only).
	Digoxin	Increased plasma concentrations of digoxin resulting from P450 inhibition may lead to increased digitalis toxicity.
	Benzodiazepines	Increased plasma concentrations of benzodiazepines, particularly midazolam, resulting from P450 inhibition may result in potentiation of the sedative effects of these drugs.
	Glipizide	Increased plasma concentrations of glipizide resulting from P450 inhibition may cause hypoglycemia.
	Second-generation antihistamines	Although identified in people, and not animals, there may be increased plasma concentrations of antihistamines resulting from P450 inhibition, which may result in cardiac arrhythmias, including ventricular tachycardia and torsades de pointes; not seen with fluconazole except at very high doses.
	Warfarin	Increased plasma concentrations of warfarin resulting from P450 inhibition may cause increased anticoagulant effects and bleeding.
	Cisapride	Although identified in people, and not animals, there may be increased plasma concentrations of cisapride resulting from P450 inhibition, which may result in ventricular arrhythmias, including torsades de pointes.
	Cyclosporine	Increased plasma concentrations of cyclosporine resulting from P450/P-gp inhibition may require adjustment of cyclosporine doses; has been used clinically to decrease the cost of cyclosporine treatment.
	Quinidine	Increased plasma concentrations of quinidine resulting from P450/P-gp inhibition may lead to increased quinidine toxicity.
	Nifedipine	Increased plasma concentrations of nifedipine resulting from P450/P-gp inhibition.
	Hydrochlorthiazide	Hydrochlorthiazide decreases the renal elimination of fluconazole, resulting in increases of fluconazole plasma concentrations.
	Carbemazapine	Induction of P450 enzymes by carbemazapine may decrease the plasma concentrations of antifungal drugs.
	Rifampin	Induction of P450 enzymes by rifampin may decrease the plasma concentrations of antifungal drugs.
	Phenytoin	Induction of P450 enzymes by phenytoin may decrease the plasma concentrations of antifungal drugs.
	Phenobarbital	Induction of P450 enzymes by phenobarbital may decrease the plasma concentrations of antifungal drugs.
	Prednisolone	Down-regulation of intestinal P-glycoprotein results in a subsequent increase in the AUC of orally administered prednisolone.
	Methadone	Inhibition of P450 enzymes results in significantly increased AUC and plasma concentrations of methadone after oral administration to healthy Greyhound dogs.
	Colchicine	Increased risk of colchicine toxicity when coadministered with azole antifungals.
	Other azole antifungals	Ketoconazole inhibits its own elimination, resulting in possible increased plasma concentrations over time.
Flucytosine	Bone marrow depressants	May exacerbate bone marrow toxicities.
	Nephrotoxic agents	May decrease flucytosine clearance, increasing the potential for bone marrow toxicity.
Terbinafine	*There are no reported drug–drug interactions with terbinafine.*	

[a] Not all of these interactions have been documented in veterinary medicine, but are present in human medicine and should be monitored for in veterinary patients.

antacids, H_2 blockers, and parietal cell proton pump inhibitors) or diseases resulting in achlorhydria will decrease its dissolution and oral absorption. Because of lack of consistent gastric acidity, ketoconazole is absorbed poorly in horses. When ketoconazole was administered at 30 mg/kg to horses in corn syrup, the drug was not detected in serum; however, when it was administered with 0.2 N hydrochloric acid intragastrically, oral absorption increased but systemic availability was only 23% with peak serum concentrations of 3.76 μg/ml (Prades et al., 1989).

Clinical Use

In people, ketoconazole has been replaced in therapy by safer triazole antifungal drugs and is no longer marketed in some countries. But in veterinary medicine, owing to ketoconazole's efficacy, safety, cost, and ease of administration, it is still a popular antifungal agent. For dermatophytosis in cats, 10 mg/kg/day has been used (Medleau and Chalmers, 1992). For candidiasis, 10 mg/kg/day for 6–8 weeks is recommended. For canine blastomycosis, histoplasmosis, cryptococcosis, and coccidioidomycosis, the dosage is 10–20 mg/kg every 12 hours. Ketoconazole may also be effective in treating nasal cryptococcosis in a dog at a dose of 10 mg/kg/day (Noxon et al., 1986). For *Malassezia* dermatitis in dogs, dosages of 5–10 mg/kg/day have been recommended (Hill et al., 1995). The duration of treatment is highly variable. Four to six weeks is a minimum for most diseases; many patients with blastomycosis are treated for a minimum of 2 months and as long as 6 months. If there is CNS involvement, particularly with cryptococcosis, higher doses (40 mg/kg) may be necessary to improve penetration into the CNS. Cats have been successfully treated for cryptococcosis with a dosage of 10–15 mg/kg/day (Pentlarge and Martin, 1986; Legendre et al., 1982; Medleau et al., 1985). As complete eradication of the fungal organism is difficult, relapse is common. For this reason, infections should be treated beyond the time clinical signs have resolved. Ketoconazole is not absorbed well orally in horses and it is not recommended. There are no approved formulations for use in food animals.

The use of ketoconazole is not limited to the treatment of fungal infections. Because of its inhibitory effect on P450 and P-glycoprotein, administration of ketoconazole concurrently with cyclosporine for the treatment of immune diseases has been used to reduce the dose of cyclosporine by up to 75% and reduce the cost of cyclosporine therapy by 58% (Dahlinger et al., 1998). A study examining blood and skin concentrations of cyclosporine with concurrent administration of ketoconazole at 2.5 mg/kg each showed this regimen to be potentially as effective as cyclosporine alone at 5.0 mg/kg for treatment of canine atopic dermatitis (Gray et al., 2013). Although this may be the most common interaction manipulated for clinical use, other drug–drug interactions have been reported. Ketoconazole (100 mg/day) administered to healthy beagle dogs resulted in down-regulation of intestinal P-glycoprotein and a subsequent increase in the AUC of orally administered prednisolone (Van der Heyden et al., 2012). Concurrent administration of ketoconazole with methadone significantly increased the AUC and plasma concentrations of methadone after oral administration to healthy Greyhound dogs (Kukanich et al., 2011). Other studies have shown that ketoconazole inhibits its own elimination, as well as that of midazolam in healthy Greyhounds, without any significant effect on fentanyl or morphine elimination (Kukanich and Hubin, 2010; Kukanich and Borum, 2008). Colchicine toxicity has also been reported to be precipitated by ketoconazole in a dog, and these drugs are not recommended for coadministration (McAlister et al., 2014).

Ketoconazole inhibits the synthesis of steroid hormones (via inhibition of the cytochrome P450 enzymes), most notably cortisol and testosterone. Although this may be a side effect of therapy, it has been exploited for the temporary management of hyperadrenocorticism in dogs (Bruyette and Feldman, 1988; Feldman et al., 1990) and as an antiandrogen treatment. Steroid synthesis inhibition is a temporary effect that persists only during dosing with ketoconazole (e.g., for up to 8 hours). Although the effects are temporary, they are effective. A recent retrospective study showed that ketoconazole administration improved clinical signs of hyperadrenocorticism in 90% of treated dogs, and 69% of dogs had cortisol concentrations following adrenocorticotropic hormone (ACTH) stimulation that were within the normal range (Lien and Huang, 2008). Dogs in that study were treated for the remainder of their life, with a median survival time after diagnosis of 25 months. Ketoconazole will not produce permanent hypoadrenocorticism.

Adverse Effects

Nausea, anorexia, and vomiting are the most common adverse effects, and may require cessation of therapy, particularly in cats (Medleau and Chalmers, 1992). They are usually dose related and may be diminished by decreasing the dose, dividing the total dose into smaller doses, and administering each dose with food. With chronic therapy pruritus, alopecia, lightening and drying of the hair coat, and weight loss may occur (Greene, 1990). Slight to moderate elevations of inducible hepatic enzymes are expected and may not be accompanied by hepatic injury. However, high elevations in hepatic enzymes, accompanied by other parameters (hyperbilirubinemia and clinical signs consistent with hepatic disease), may indicate hepatotoxicosis. Idiosyncratic hepatitis has been reported in animals and people (Janssen and Symoens, 1983).

Drug Interactions

Ketoconazole is a very potent inhibitor of fungal P450, but it also inhibits mammalian P450 at relatively low concentrations (Aidasani et al., 2008); therefore, side effects and drug interactions can occur. Inhibition of P450(17α) catalyzed conversion of progestins to androgens occurs during treatment. Dose-related inhibition of testosterone has resulted in gynecomastia, sexual impotence, and azoospermia. Cats appear to be more sensitive to ketoconazole liver toxicity than are dogs but they are less sensitive to the hormonal suppressive side effects (Willard et al., 1986a, 1986b).

Ketoconazole has been shown to be teratogenic in the rat and has resulted in mummified fetuses and stillbirths in dogs. It is therefore not recommended for use in pregnant or lactating animals. Cataracts have been reported after long-term ketoconazole therapy in dogs (de Costa et al., 1996). The average duration of therapy in affected dogs was 15 months, and dosages ranged from 6 to 31 mg/kg/day. These dogs were not diabetic. The mechanism of this reaction is not known.

Fluconazole

Fluconazole (Diflucan® and generic) has replaced ketoconazole in small animals and birds for many indications. The triazole groups result in increased resistance to metabolic attack, in vivo potencies 100 times that of ketoconazole, and significantly increased aqueous solubility (8 mg/ml) (Richardson et al., 1990). Because of these properties, this compound has good efficacy in animal models and pharmacokinetic properties that are improved over other azole antifungal drugs such as ketoconazole or itraconazole. It is available in 50–200 mg tablets, powder for oral suspension, and a 2 mg/ml parenteral formulation. Compounded formulations have good oral bioavailability and can be used with a reasonable expectation of performance.

Spectrum of Activity

Fluconazole has been shown to be effective for animal infections caused by *Blastomyces, Candida, Coccidioides, Cryptococcus*, and *Histoplasma*. It is not particularly active against *Aspergillus*. Resistant *Aspergillus* strains have been increasing in human medicine with MICs often >256 μg/ml. For this reason, fluconazole should not be used as a first choice for the treatment of aspergillosis unless susceptibility has been determined. Efficacy of fluconazole in people has been associated with AUC/MIC ratios as being the best predictor of cures. A ratio above 25 is considered desirable for the best outcome (Goodwin and Drew, 2008).

Pharmacokinetics

Fluconazole has different solubility characteristics than ketoconazole and itraconazole and is absorbed well regardless of the circumstances. Feeding or formulation (liquid versus tablet) does not affect absorption. Fluconazole demonstrates linear absorption kinetics, with bioavailability greater than 90% in most species (Brammer et al., 1990); thus, oral and IV dosages are identical. Maximum fluconazole concentrations are reached 1–4 hours after an oral dose. Unlike other azole antifungals, fluconazole is not highly protein bound. Humphrey et al. (1985) found plasma protein binding to be between 10 and 12% at concentrations of 0.1 and 1 μg/ml in mice, rats, dogs, and humans. Similar protein binding has also been documented in horses (12.3%) at a concentration of 5 μg/ml. Fluconazole's low molecular weight, water solubility, and high unbound fraction allow it to be readily distributed throughout the body, including privileged spaces that ordinarily exclude many drugs. Drug concentrations in saliva, sputum, skin, nails, blister fluid, and vaginal tissue and secretions were found to be similar to plasma concentrations. The advantages of fluconazole lie in its ability to produce higher CSF concentrations than ketoconazole or itraconazole; therefore, it may be useful for treating mycotic meningitis (Kowalsky and Dixon, 1991). Fluconazole CSF/plasma or CSF/serum concentration ratios range from 0.49 in horses (Latimer et al., 2001) to 0.88 in cats (Vaden et al., 1997). The drug also penetrates well into the aqueous humor with ratios of aqueous : plasma of 0.37 and 0.79 in the horse and cat, respectively.

Fluconazole is eliminated principally by the kidney. A unique feature of fluconazole is that this drug is the only one of the azoles that is water soluble and excreted in the urine in an active form; therefore, it may be one of the few drugs useful for treating fungal cystitis. As can be expected with a renally excreted drug, renal dysfunction affects fluconazole's elimination such that dose adjustments are necessary. When patients with normal kidney function were compared with those with severe renal insufficiency, fluconazole's elimination half-life nearly tripled (from 30.1 hours to 84.5 hours) (Dudley, 1990). Reduced dosages as well as extended dosing intervals have been recommended for patients with chronic kidney disease. The disparity between renal fluconazole clearance and creatinine clearance suggests that net tubular reabsorption is responsible for the extended half-life. Half-life was measured to be approximately 14 hours in dogs, 13–25 hours in cats (Vaden et al., 1997; Craig et al., 1994), and 38 hours in horses (Latimer et al., 2001). Steady-state concentrations are achieved in 5–7 days; thus, the manufacturer suggests a two-times loading dose during the first 12–24 hours (Dudley, 1990). The lack of significant hepatic metabolism allows for linear elimination kinetics; that is half-life is independent of dose.

Clinical Use

Small animals: Fluconazole is most often used for treatment of dermatophytes. Although not as active against

Aspergillus or *Penicillium* as other azoles, it has also been used to treat canine nasal aspergillosis and penicilliosis. Ten affected dogs were treated with 2.5–5 mg/kg/day fluconazole orally for 8 weeks. Six dogs became free of disease 2–4 weeks after cessation of therapy and remained free of disease for at least 6 months. Serum alkaline phosphatase and alanine transaminase activity remained within normal ranges throughout the treatment period, and adverse side effects were not noted (Sharp, 1991). Doses as high as 10–12 mg/kg/day have also been recommended in dogs. Fluconazole is also at least as effective as ketoconazole for the treatment of dogs with *Malassezia* dermatitis (Sickafoose et al., 2010). Fluconazole is associated with survival to clinical remission in 75% of dogs with blastomycosis, which was not statistically different than the 90% survival with itraconazole (Mazepa et al., 2011). The cost of fluconazole therapy in that study was approximately one-third that of itraconazole, and both drugs caused a similar incidence of hepatotoxicosis (elevated alanine aminotransferase, ALT). For cats with systemic cryptococcosis, clinical studies have shown a benefit from a dose of 100 mg/cat/day in one or two divided doses. A practical dose is one 50-mg tablet per cat, once a day, or twice daily for refractory cases. Other reported doses are in the range of 2.5–5 mg/kg once a day (Hill et al., 1995). Pharmacokinetic studies support a dose of 50 mg/cat per day for nasal or dermal cryptococcosis (Vaden et al., 1997).

Exotic animals: The doses for exotic animals are listed in Table 38.4. The half-life can be prolonged in reptiles because of the dependence on renal elimination. Thus, the half-life was 138 hours in turtles when injected SC, which allows for treatment once every 5 days (Mallo et al., 2002).

Large animals: Oral absorption in horses is reported to be greater than 100% (Latimer et al., 2001). From this cited study a dosing regimen of a loading dose of 14 mg/kg orally, followed by 5 mg/kg q 24 h was derived for horses to produce sufficient concentrations in plasma and tissues. This dose has been successful in treating cryptococcal meningitis and optic neuritis (Hart et al., 2008), and nasal conidiobolomycosis lesions in adult horses (Taintor et al., 2004) as well as disseminated candidiasis in foals (Reilly and Palmer, 1994).

Adverse Effects

Fluconazole has been generally well tolerated, with mild adverse effects being reported in 5–30% of cases. The GI tract was most frequently involved, followed by the CNS and skin. Elevations in hepatic enzymes have been observed, in small animals and horses, sometimes necessitating termination of treatment. Hematological abnormalities, including anemia, leukopenia, neutropenia, and thrombocytopenia, have been reported in people. In subacute toxicity studies in dogs, the highest dose tested (30 mg/kg) caused slight increases in liver weight, hepatic fat, and plasma transaminase activity. Although there is no evidence of mutagenicity or carcinogenicity, its use in pregnant patients is not recommended. However, it has been used successfully with no observed adverse effects on the fetus in horses in the seventh and tenth month of gestation (Taintor et al., 2004).

Drug Interactions

Drug–drug interactions are less frequently reported with fluconazole than other azole antifungals; however, they do occur. Fluconazole has been shown to significantly increase cyclosporine concentrations in both normal and renal transplanted dogs (Katayama et al., 2008, 2010a). Treatment with fluconazole has been shown to significantly prolong anesthesia times in horses following induction regimens that include midazolam (Krein et al., 2014). Compared to ketoconazole, there is little evidence of testosterone or other steroid biosynthesis inhibition (Shaw et al., 1987) in human or animal patients (VanCauteren et al., 1987b).

Itraconazole

Itraconazole (Sporanox®) was approved for use in the United States in 1992. Of several triazole compounds screened, itraconazole, first synthesized in 1980, was selected for further clinical development due to several criteria: (i) 5–100 times better in vitro and in vivo potency than ketoconazole, (ii) good activity against *Aspergillus* spp., (iii) activity against meningeal cryptococcosis in animal models, (iv) fewer adverse effects compared to ketoconazole, and (v) favorable pharmacokinetics (Cauwenbergh et al., 1987).

Itraconazole is a weak base (pK_a = 3.7), is highly lipophilic (logP = 5.66), and is practically insoluble in water. There are several different formulations available. The intravenous formulation is rarely used in veterinary medicine due to expense as well as instability once reconstituted. There are three oral formulations available. The oral capsules were the first formulation marketed and they are still commonly used. They are available in 100 mg dose strength, and consist of drug coated onto small sugar spheres. The capsules require an acid environment for dissolution and therefore absorption is often highly variable. There is also an oral solution approved for use in humans that contains 10 mg/ml of itraconazole complexed with hydroxypropyl-β-cyclodextrin, to increase the solubility. This product has been demonstrated to have higher, less variable absorption in humans, cats, and horses (Willems et al., 2001; Boothe et al., 1997; Davis et al., 2005; Mawby et al., 2016) but is bioequivalent to the capsules in dogs

Table 38.4 Selected systemic antifungal drugs used in exotic animal species

Species	Drug	Disease treated	Dosing protocol	Reference
Passerine and Softbill Birds	Fluconazole	Candidiasis	2–5 mg/kg PO q24h for 7–10 days	Dorrestein, 2000
	Griseofulvin	Dermatophytosis	20 mg/kg PO q24h for 4–6 weeks	
	Itraconazole	Aspergillosis	5–10 mg/kg PO q12–24h in orange juice or 0.1N HCl for 14 days	
	Ketoconazole	Dermatophytosis	20–30 mg/kg PO q12h for 14–30 days	
	Miconazole	Candidiasis or cryptococcosis	10–20 mg/kg IM or IV q8–24h	
	Nystatin	Intestinal candidiasis	100,000 IU/l of drinking water or 200,000 IU/kg soft food for 3–6 weeks	
Psittacine Birds	Fluconazole	Candidiasis	2–5 mg/kg PO q24 h for 7–10 days	Tully, 2000
	Flucytosine	Aspergillosis	60–150 mg/kg PO q12h in adults; 100–250 mg/kg PO q12h in neonates. Usually given in combination with amphotericin B	
	Itraconazole	Aspergillosis	5–10 mg/kg q12h for 4–5 weeks; 5 mg/kg q24h in African grays	
	Ketoconazole	Candidiasis	20–30 mg/kg q12h in orange or pineapple juice, lactulose, or methylcellulose for 14–30 days	
	Voriconazole	Aspergillosis	12–18 mg/kg oral q12h	Flammer et al., 2008
	Miconazole	Candidiasis or cryptococcosis	20 mg/kg IV q8h	
	Nystatin	Intestinal candidiasis	100,000–300,000 IU/kg PO q8–12h	
Raptors	Fluconazole	Mycelial candidiasis, systemic mycosis	5–15 mg/kg PO q12h for 14–60 days	Huckabee, 2000
		Gastrointestinal and systemic candidiasis	2–5 mg/kg PO q24h for 7–10 days	
	Flucytosine	Aspergillosis	120 mg/kg PO q6h; 20–30 mg/kg PO q6h for 60–90 days; 50–75 mg/kg PO q8h in combination with amphotericin B	
		Candidiasis	250 mg/kg PO q12h	
	Itraconazole	Aspergillosis	15 mg/kg PO q12h for 4–6 weeks	
	Ketoconazole	Candidiasis	15 mg/kg PO q12h	
		Aspergillosis	30–60 mg/kg PO q12h for 14–30 days	
	Nystatin	Intestinal candidiasis	100,000–300,000 IU/kg PO q8–12h	
Pet Fish	Itraconazole	Systemic mycoses	1–5 mg/kg q24h in feed for 1–7 days	Mashima and Lewbart, 2000
	Ketoconazole	Systemic mycoses	2.5–10 mg/kg PO, IM or ICe	
Ferrets	Amphotericin B	Systemic mycoses	0.4–0.8 mg/kg IV once a week to a total dose of 7–25 mg	Williams, 2000
	Griseofulvin	Dermatophytosis	25 mg/kg PO q24h for 3–4 weeks	
	Ketoconazole	Systemic mycoses	10–30 mg/kg PO q12h–24h	
Hedgehogs	Griseofulvin	Dermatophytosis	25–50 mg/kg PO q24h	Lightfoot, 2000
	Itraconazole	Systemic mycoses	5–10 mg/kg PO q12–24h	
	Ketoconazole	Systemic yeast/fungal infections	10 mg/kg PO q24h	
	Nystatin	Yeast infections	30,000 IU/kg PO q8–24h	
Marsupials	Griseofulvin	Dermatophytosis	20 mg/kg PO q24h for 30–60 days	Johnson-Delaney, 2000
	Nystatin	Candidiasis	5000 IU/kg q8h for 3 days	

(*continued*)

Table 38.4 (*Continued*)

Species	Drug	Disease treated	Dosing protocol	Reference
Rabbits	Amphotericin B	Systemic mycoses	1 mg/kg IV q24h	Ivey and Morrisey, 2000
	Fluconazole	Systemic mycoses	25–43 mg/kg slow IV q12h	
	Griseofulvin	Dermatophytosis	12.5–25 mg/kg PO q12–24h for 10–42 days	
	Ketoconazole	Dermatophytosis	10–40 mg/kg PO q24h for 14 days	
Rodents	Amphotericin B	Candidiasis	0.43 mg/kg PO or 0.11 mg/kg SC in mice	Adamcak and Otten, 2000
	Griseofulvin	Dermatophytosis	25 mg/kg PO q24h for 14–28 days in gerbils, guinea pigs, hamsters, and rats; 14 days in mice; 28–40 days in chinchillas	
	Itraconazole	Systemic mycoses	5 mg/kg PO q24h in guinea pigs; 50–150 mg/kg q24h in mice; 2.5–10 mg/kg PO q24 in rats	
	Ketoconazole	Systemic mycoses/candidiasis	10–40 mg/kg PO q24h for 14 days in all species	
Amphibians	Amphotericin B	Systemic mycoses	1 mg/kg ICe q24h for 14–28 treatments	Walker and Whitaker, 2000
	Fluconazole	Systemic mycoses	60 mg/kg PO q24h for 7 days	
	Itraconazole	Superficial and systemic mycoses	2–10 mg/kg PO q24h for 14–28 days	
	Ketoconazole	Systemic mycoses	10–20 mg/kg PO q24h for 14–28 days	
Chelonians	Amphotericin B	Aspergillosis	1 mg/kg ICe q24h for 2–4 weeks	Bonner, 2000
	Griseofulvin	Dermatophytosis	20–40 mg/kg PO q72h for 5 treatments	
	Itraconazole	*Sceloporus* sp.	20–30 mg/kg PO q6–8h in the spiny lizard	
	Ketoconazole	Systemic mycoses	25 mg/kg PO q24h for 2–4 weeks in turtles; 15 mg/kg PO q24h (27°C) in the gopher tortoise	
	Nystatin	Enteric fungal infections	100,000 IU/kg PO q24h for 10 days in turtles	
Reptiles	Amphotericin B	Systemic mycoses	1 mg/kg IT q24h for 14–28 days	Funk, 2000
	Fluconazole	Systemic mycoses	2–5 mg/kg PO q24h for 5–21 days in lizards and snakes; can also mix 100 mg with 20 ml of nystatin and give PO at 0.5–0.6 ml/kg	
	Fluconazole		For sea turtles, a loading dose of 21 mg/kg, followed by 10 mg/kg every 5 days, injected SC (Mallo et al., 2002)	
	Griseofulvin	Dermatophytosis	20–40 mg/kg PO q72h for 5 treatments in snakes	
	Ketoconazole	Superficial and systemic mycoses	25 mg/kg PO q24h for 3 weeks in snakes	
	Nystatin	Enteric fungal infections	100,000 IU/kg PO q24h	

(Hasbach et al., 2017). In cats, the oral solution was absorbed five times higher than the oral capsule (Mawby, Whittemore, and Papich unpublished data). A third oral formulation is licensed for use in cats in the United States (Itrafungol, Elanco). This formulation is also an oral solution (10 mg/ml itraconazole) with similar solubilizing agents and excipients as the human formulation.

Spectrum of Activity

Itraconazole has been tested both in vitro and in vivo against a wide variety of fungi (for review see Perfect et al., 1986; VanCutsem et al., 1987; VanCutsem, 1990; Cauwenbergh and DeDonker, 1987). It was found to be effective against virtually all medically important fungi, including *Microsporum, Trichophyton,* *Candida, Malassezia, Sporothrix, Pythium, Histoplasma, Aspergillus, Blastomyces, Coccidioides*, and *Cryptococcus*. It has little activity against *Fusarium* sp.

Like other azole antifungal drugs, the AUC/MIC is the best surrogate marker to predict efficacy. However, the most often reported drug concentrations from studies in humans have been the plasma concentrations measured at the lowest point (trough, or C_{min}) during multiple dosing. In these studies (Goodwin and Drew, 2008) the trough concentrations greater than 0.5 to 1.0 μg/ml have been associated with clinical success.

Pharmacokinetics

Absorption is increased by an acid environment and when taken with meals and is less variable than ketoconazole absorption. Bioavailability increases from 40% after fasting to 99.8% when given with a meal (VanCauteren et al., 1987a), except in horses. Due to the low solubility of itraconazole, commercially available formulations include solubility enhancers. Without these specialized formulations, absorption is negligible. Comparison of the oral absorption of Sporanox® capsules, generic and compounded itraconazole capsules showed that, in dogs, the formulations are not bioequivalent (Mawby et al., 2014). Although therapeutic concentrations are reached with the generic formulations, relative bioavailability of the compounded capsules is only approximately 5%. The compounded suspension and capsule had negligible oral absorption in cats (Mawby et al., 2016). This feature of itraconazole also has been demonstrated in other species as well, including birds and horses. Thus, the use of compounded itraconazole formulations is not recommended.

Itraconazole is highly (99.8%) protein bound (95% to albumin and 5% to red blood cells) (Heykants et al., 1987); however, due to its lipophilicity and even higher affinity for tissue proteins, it is extensively distributed throughout the body. Tissue to plasma concentration ratios range from 1 : 1 in brain to 8 : 1 in keratin to 25 : 1 in fat stores. Highest tissue levels are seen in the liver and adrenal cortex (Heykants et al., 1987). High tissue binding also produces a very large volume of distribution (Troke et al., 1990; Heykants et al., 1990) and low plasma concentrations. Although it does not reach high concentrations in the CSF compared to fluconazole, itraconazole was found to be effective in treating meningeal cryptococcosis in both mouse and guinea pig models (Perfect et al., 1986).

Itraconazole is extensively metabolized, with less than 1% of the active drug and approximately 35% of inactive drug (as more than 10 metabolites) excreted in the urine. The major metabolite, hydroxyitraconazole, has similar antifungal activity to the parent drug, and is often found at concentrations two to three times higher than itraconazole in the plasma in humans (Willems et al., 2001). The metabolite to parent drug ratio is reported to be similar in dogs (Yoo et al., 2002); however, this metabolite has not been found in either cats (Itrafungol®, package insert) or horses (Davis et al., 2005). The predominant route of elimination for itraconazole is in the bile. Because of the increased metabolic stability of the triazole ring versus the imidazole ring (Richardson et al., 1990), itraconazole has a longer half-life (17–25 hours) than ketoconazole (8 hours) in humans. There is disagreement about the elimination rate since the terminal half-life in the dog has been reported to be 8–12 hours (VanCauteren et al., 1987a) and 44–58 hours (Heykants et al., 1987). Differences in study methods, assay sensitivity, and pharmacokinetic analysis may account for this discrepancy. More important than plasma half-life, therapeutically active concentrations are maintained much longer in tissues than in plasma. For example, itraconazole can be detected for 4 days in vaginal epithelium and for 4 weeks in skin and nails after cessation of therapy. These long-lasting tissue concentrations account for the ability to administer this drug intermittently for some fungal infections, as will be discussed below in Section Clinical Use. Itraconazole, like ketoconazole, exhibits nonlinear pharmacokinetics; steady-state concentrations were found to be three times higher after 14 days of therapy than those predicted by a single dose, and the half-life was seen to increase from 24 to 36 hours (Heykants et al., 1990).

Pertinent to the pharmacokinetics is the ability of itraconazole to inhibit drug metabolizing enzymes. Itraconazole and its metabolites are cytochrome P450 inhibitors (Templeton et al., 2008). Metabolizing enzymes may be saturated producing nonlinearity in elimination. In addition, repeated dosing may produce a time-dependent decrease in clearance and accumulation (Templeton et al., 2008). Therefore, with repeated dosing, the clearance may decrease and half-life increase. Coadministration of itraconazole and cyclosporine has been shown to result in increased cyclosporine concentrations in cats (Katayama et al., 2010b).

Clinical use

Small animals: Itraconazole is one of the most commonly administered oral antifungal agents for small animals. It has no endocrine effects compared to ketoconazole and is better tolerated. Itraconazole is highly bound in plasma and there is strong binding to keratin producing drug concentrations in skin that persist 2–4 weeks after cessation of drug therapy. It may be excreted into the sebum, increasing the concentrations in skin. This allows for pulse dosing for some diseases. *Histoplasma, Cryptococcus*, and *Blastomyces* are highly susceptible; *Candida, Aspergillus,* and *Penicillium* are less sensitive. Itraconazole also has been used to treat cutaneous leishmaniasis because the *Leishmania* organism has ergosterol in high concentrations in its cell wall.

Cats: Itraconazole is probably better tolerated in cats than ketoconazole. Nevertheless, adverse effects are still possible. Since most adverse effects are dose related, one is advised to lower the dose in animals in which adverse effects are observed. One report indicated that there were dose-related GI effects of anorexia and vomiting in cats from administration of itraconazole (Mancianti et al., 1998).

The dosing regimens for cats were reviewed by Moriello (2004). Doses in cats vary from 5–10 mg/kg once a day, orally for at least 56 days, to 10 mg/kg once a day, for 28 days, followed by pulse therapy of 1 week on/1 week off. Lower doses of 1.5 to 3 mg/kg once daily for cycles of 15 days at a time are also used. The most recent regimen to be studied is 100 mg capsule per cat every other day for up to 8 weeks. This regimen yielded average therapeutic trough plasma concentrations (>0.5 μg/ml) within 3 weeks, however two of the ten cats in the study developed reversible adverse effects (Middleton et al., 2016).

The availability of a commercial form for cats has helped to define the use in this species. As mentioned previously, itraconazole (Itrafungol®) 10 mg/ml oral solution is registered for use in cats to treat dermatophytosis (not registered in the USA). The treatment schedule consists of once-daily doses of 5 mg/kg for three 1-week cycles. After each week of treatment, it should be followed by a week without treatment (week on/week off schedule). This schedule has been evaluated in cats and maintains drug concentrations in hair during the non-treatment phase (Vlaminck and Engelen, 2004).

Itraconazole has been compared to ketoconazole, with each drug administered at doses of 10 mg/kg/day for the treatment of experimentally induced feline disseminated cryptococcosis (Medleau et al., 1990). After 3 months of therapy, the infection had been cleared by both drugs as determined by cryptococcal antigen titers and CSF culture. Three months following therapy all animals remained clinically normal, and titers and CSF cultures remained negative. Although both antifungals brought about resolution of the disease, all cats receiving ketoconazole became anorectic and lost weight, requiring dosage adjustments. This was not seen with itraconazole, and in fact the animals receiving this drug gained weight during the study. Itraconazole has also been used in naturally occurring cryptococcal infections (Medleau, 1990), where an increase in treatment failure was noted in cats that were seropositive for FIV or feline leukemia virus (FeLV).

Dogs: In dogs, the most extensive study has been for treatment of blastomycosis (Legendre et al., 1996). In a study of 112 dogs, 5 mg/kg/day was as effective as 10 mg/kg/day. With a 60-day course of therapy, 54% of dogs were cured. Itraconazole has been used to treat ocular and systemic blastomycosis in dogs. When given 5 mg/kg itraconazole twice a day for 60 days, 76% of eyes with posterior segment disease other than optic neuritis and 18% and 13% of eyes with anterior uveitis or endophthalmitis, respectively, recovered (Brooks et al., 1992). Pulse dosing has also been evaluated in dogs. Itraconazole doses of 5 mg/kg PO q 24 h for 2 consecutive days per week for 3 weeks was found to be as effective as a dose of 5 mg/kg PO q 24 h for 21 consecutive days in the treatment of *Malassezia* dermatitis and otitis (Pinchbeck et al., 2002).

Itraconazole has been successfully used in both the prevention and the treatment of aspergillosis in caged birds. A dose of 20 mg/kg daily for at least 30 days was used to successfully treat five of 12 presumed cases of *Aspergillus* infections in penguins. This same author suggests its prophylactic use in penguin chicks (Shannon, 1992). A different treatment protocol was recommended for aspergillosis in raptors. Birds are treated with 10 mg/kg twice daily in combination with amphotericin B nebulization three times a day for 20 minutes. Treatment for some cases lasted as long as 6 weeks. These authors also recommend the prophylactic use of itraconazole whenever the clinician expects increased risk for the disease (Forbes et al., 1992). Other antifungal dosing regimens in birds and other exotic animal species are listed in Table 38.4.

Large animals: Itraconazole has been reported to be effective in horses for the treatment of mycotic rhinitis, osteomyelitis, and guttural pouch mycosis (Korenek et al., 1994; Foley and Legendre, 1992; Davis and Legendre, 1994). A pharmacokinetic study showed that the oral solution at a dose of 5 mg/kg q 24 h will produce adequate levels in blood and tissues for successful treatment (Davis et al., 2005). However, the use of the oral liquid will require large volumes — most likely requiring intragastric administration in horses — and the drug is very expensive. The oral capsules have a lower bioavailability and higher doses or more frequent dosing intervals are recommended. There are no reports of the use of this drug in food animals, and there are no approved formulations for these species.

Adverse Effects

Itraconazole is up to 125 times more selective for fungal P450 systems than mammalian liver enzymes in certain in vitro preparations (Vanden Bossche, 1987). It also does not inhibit P450 systems in the testis, adrenal, or liver in vivo (Vanden Bossche et al., 1990). In clinical studies, 100 mg of itraconazole given to humans each day for 30 days had no effect on serum testosterone or cortisol levels (DeCoster et al., 1987). Similarly, there were no changes in testosterone and cortisol concentrations in rats and dogs receiving daily itraconazole for at least 1 month.

The biochemical basis for the specificity of itraconazole toward fungal P450 is thought to be dependent upon the hydrophobic nonligand portion of the molecule and its affinity for the apoprotein portion of the cytochrome molecule (Vanden Bossche et al., 1990). The resulting lack of significant inhibition of liver microsomal enzymes results in itraconazole's inability to affect other drugs' metabolism. Although the clinical significance is as yet unknown, drugs that can inhibit or stimulate liver degradative enzymes are able to alter the pharmacokinetics of itraconazole. Even though itraconazole is primarily cleared by hepatic metabolism, there appears to be no need for dosage adjustments in patients with liver disease (Heykants et al., 1987). As with ketoconazole, itraconazole's oral absorption is pH dependent; therefore, dosage adjustments may be necessary when gastric pH is increased.

Because itraconazole is better tolerated than ketoconazole, it is used as the drug of choice for long-term treatment. Dogs, cats, and exotic and zoo animals have received this drug for weeks without adverse effects. The capsules have been administered for up to 6 months in horses with no reported adverse effects. Nevertheless, adverse effects are still possible. Since most adverse effects are dose related, one is advised to lower the dose in animals in which adverse effects are observed. According to Legendre (1995) about 10% of dogs receiving recommended doses develop hepatic toxicosis. Liver enzyme elevations may occur in 10–15% of dogs. Itraconazole has been well tolerated by clinically ill cats, although one case of fatal drug-induced hepatitis has been reported (Medleau, 1990). Anorexia may occur as a complication of treatment, especially with high doses and high serum concentrations. It usually develops in the second month of therapy in dogs. In cats there seem to be dose-related GI effects of anorexia and vomiting (Mancianti et al., 1998). Drug-related cutaneous vasculitis has also been reported as a complication of itraconazole therapy in dogs (Nichols et al., 2001).

Dogs chronically administered itraconazole (2.5, 10, or 40 mg/kg daily for 3 months) had no significant alterations in mortality rate, behavior, appearance, food consumption, body weight, hematological values, serum and urine chemistry, or gross pathology (VanCauteren et al., 1987b). Subacute toxicity studies in rats revealed increased adrenal gland weight and the accumulation of proteinaceous material in the mononuclear phagocyte system at doses of 40 and 160 mg/kg. Since the mononuclear phagocyte system is responsible for clearing the host of a fungal infection, the clinical importance of this toxic effect is undetermined. Although not teratogenic at 10 mg/kg, maternal toxicity, embryo toxicity, and teratogenicity were observed at 40 and 160 mg/kg in rats (VanCauteren et al., 1987b); therefore, its use in pregnant animals is not recommended.

Postmarketing drug monitoring in humans has shown that itraconazole may cause or exacerbate underlying heart conditions. There is a dose-related negative inotropic effect seen in both healthy human volunteers and in anesthetized dogs. Owners should therefore be counseled to monitor the patients for signs of heart failure and to discontinue the drug if clinical signs are observed.

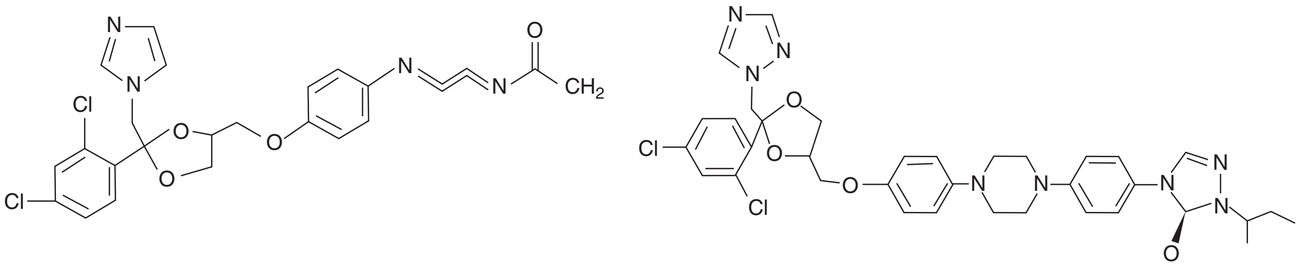
Mild to moderate kidney disease has not been reported to change the pharmacokinetic clearance of itraconazole in humans. However, if the intravenous formulation or the oral Sporanox® solution is used, renal failure may decrease the elimination of the carrier molecule hydroxypropyl-β-cyclodextrin. Therefore, the oral capsules are recommended for use in these patients.

Voriconazole

The newest triazole to be investigated in animals is voriconazole (Vfend®). Voriconazole is similar in structure to fluconazole (Figure 38.4); however, the substitution of a fluoropyrimidine ring for one of the triazole moieties and the additional of a methyl group to the propanol backbone increases the spectrum of activity and potency as well as the fungicidal activity against some species of molds, including *Aspergillus* and *Fusarium* spp. In a survey of fungal pathogens, voriconazole inhibited greater than 95% of *Aspergillus* with a concentration less than or equal to 1 μg/ml (Diekma et al., 2003). It is more lipophilic than fluconazole, more water soluble than itraconazole or ketoconazole, with intermediate protein binding. These properties allow for excellent oral bioavailability and tissue distribution. In people, the plasma concentrations were highly variable among individuals, which is caused by variations in hepatic metabolism, other medications coadministered, and nonlinear elimination. The pharmacodynamic parameters associated with clinical cure are an AUC/MIC plasma concentration of 20–25, or a plasma concentration above 2 μg/ml.

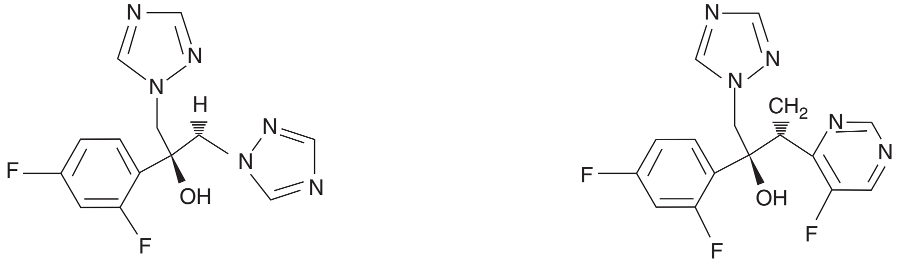
Pharmacokinetics

The pharmacokinetics of voriconazole have been investigated in dogs, horses and avian species, with preliminary data available for llamas and cats. Across species, the pharmacokinetics vary widely. Experimental studies in dogs have shown rapid and complete absorption of the drug following oral administration (Roffey et al., 2003). Half-life is short (approximately 3 hours), and at a dose of 6 mg/kg/day orally, plasma concentrations remained above the target MIC of 1 μg/ml for only 15 hours and the target AUC : MIC ratio of 20–25 for free drug concentrations were not reached (Lemetayer et al., 2015).



Ketoconazole

Itraconazole



Fluconazole

Voriconazole

Figure 38.4 Chemical structures of commonly used systemic azole antifungal drugs.

Although the authors of that study state twice-daily dosing may be necessary, an effective dose cannot be extrapolated from current data as voriconazole exhibits nonlinear pharmacokinetics in this species, with a ninefold increase in plasma concentrations seen following a fourfold increase in dose (Lemetayer et al., 2015; Roffey et al., 2003). Interestingly, autoinduction of drug metabolism has been shown to occur in dogs, further complicating dosing recommendations as an increase in dose may be required with multiple administrations. Although drug was detected in body fluids, including CSF, aqueous humor and synovial fluid, it was found at a lower percentage than other reported species (Lemetayer et al., 2015).

Whereas the metabolism appears to increase with repeated doses in dogs causing lower concentrations after multiple doses, the opposite phenomenon occurs in cats. In the studies by Vishkautsan et al. (2016), the half-life was much longer in cats than dogs. The IV half-life was 12.4 hours, but after oral administration of 4–6 mg/kg, the half-life was 43 hours (± 9.02) producing an inflated oral absorption of 264%. Moreover, in the multiple dosing study of 25 mg per cat loading dose followed by 12.5 mg per cat every 48 hours, accumulation was observed steadily to 14 days and steady state was not achieved.

The pharmacokinetics of voriconazole have been studied following single and multiple dose administration in the horse (Davis et al., 2006; Colitz et al., 2007). In these cited equine studies, voriconazole had excellent oral absorption (95% and 100%) and a moderate half-life (8–13 hours) following oral administration. The oral dose used in the study by Davis et al. (2005) was 4 mg/kg and produced plasma concentrations higher than necessary for the treatment of most common veterinary pathogens, with the exception of *Fusarium* sp. The study by Colitz et al. (2007) used 3 mg/kg orally twice daily and produced concentrations above the minimum level necessary for successful treatment. When administered at 4 mg/kg orally once a day for 2 weeks in nonfasted horses, there was no statistically significant difference between voriconazole concentrations in plasma and body fluids when comparing days 7 and 14, suggesting enzyme induction may not be prominent in this species (Passler et al., 2010). In horses, voriconazole has good distribution into the aqueous humor, CSF, peritoneal fluid, pulmonary epithelial lining fluid, synovial fluid, urine, and periocular tear film. The concentrations in plasma, tissues, and other fluids exceed the minimum concentration recommended for successful therapy (Goodwin and Drew, 2008).

After oral administration of single doses ranging from 6 to 18 mg/kg in African grey parrots, a follow-up study examined multiple doses at the highest dose (18 mg/kg every 12 hours for 9 days). Compared to mammals, the elimination half-life was short at 1.1 to 1.6 hours (Flammer et al., 2008). A similar study looked at 18 mg/kg q 8 h for 11 days in Hispaniolan Amazon parrots, with similar results (Sanchez-Migallon Guzman et al., 2010). With multiple doses the kinetics changed, suggesting induction of the hepatic metabolism as mentioned for dogs above, and requiring dose adjustment for long-term treatment. Polyuria was observed in the treated birds, but no other adverse reactions were reported.

A single dose pharmacokinetic study is published on voriconazole in alpacas (Chan et al., 2009). Oral absorption is low in this species, and high daily doses would likely be needed for treatment success.

Clinical Use

Clinical experience with this drug is currently limited due to potential adverse effects, as well as cost. Successful reports of treatment of miscellaneous fungal infections are available, including intracranial phaeohyphomycotic granuloma and *Exophilia dermatitidis* in dogs, and pulmonary aspergillosis in a foal (Bentley et al., 2011; Murphy et al., 2011; Hilton et al., 2009). In these species, the most common clinical use of voriconazole is as a topical or local ocular therapy. Topical administration of the commercially available intravenous voriconazole solutions diluted to 1%, has good ocular penetration through an intact cornea with little to no local irritation (Clode et al., 2006). Intracorneal administration of 5% voriconazole solution has also been reported to result in resolution of clinical disease, specifically stromal fungal abscessation and secondary uveitis (Smith et al., 2014). At this time, the use of voriconazole in cats cannot be recommended.

Birds: The experience with voriconazole in birds has been reported by Flammer et al. (2008). On the basis of this study, the authors concluded that 12–18 mg/kg orally twice daily would be sufficient for treatment of some *Aspergillus* infections; higher doses may be needed for some infections and to maintain concentrations during long-term treatment.

Adverse Effects

Voriconazole appears to be safe for use in horses following multiple doses. The only adverse event reported in this species was in a single horse that developed pruritis after drug administration; however, these signs were controlled with administration of an antihistamine 2 hours prior to dosing (Passler et al., 2010). Except for the polyuria observed in birds, the drug also appears to be safe in the avian species studied. In dog studies using multiple doses, several dogs showed mild to moderate gastrointestinal disturbances (loss of appetite and diarrhea) and one dog had mild increases in liver enzymes (Lemetayer et al., 2015). Intravenous injection of 10 mg/kg in dogs results in severe, acute toxicity.

Cats appear to be the most susceptible to adverse drug events caused by voriconazole administration. Adverse effects in cats involving the gastrointestinal tract, eyes, and neurological function have been reported (Quimby et al., 2010; Smith and Hoffman, 2010; Vishkautsan et al., 2016). Inappetance, lethargy, and weight loss are common. Ataxia and hindlimb paresis that resolved following withdrawal of drug administration were noted in several cats. One proposed mechanism for the neurotoxicity associated with azole antifungals is through inhibition of voltage-gated calcium channels in neuronal cells (Heusinkveld et al., 2013). Visual abnormalities noted include mydriasis, decreased to absent pupillary light responses, and decreased menace response. On the other hand, in the study by Vishkautsan et al. (2016), miosis occurred from an unknown mechanism. Also noted in that study was excess salivation from administration of the oral suspension, but not from the oral tablet. Azotemia has also been noted, although it may be attributable to dehydration secondary to GI disturbances, or concurrent NSAIDs administration, rather than voriconazole having a direct nephrotoxic effect. One cat was also described to have a cutaneous drug reaction. In the case reports involving cats, they were administered doses that were similar to the canine dose, or a dose extrapolated from people (i.e., up to 13 mg/kg per day). As the study by Vishkautsan et al. (2016) showed, these doses are probably too high because of the slow clearance of voriconazole in cats compared to other animals. Prolonged QT intervals and arrhythmias have been reported in both dogs and cats. Because of adverse effects in people, it is recommended that in humans doses be adjusted to produce trough concentrations no higher than 4–6 μg/ml in order to avoid toxicity (Ashbee et al., 2014).

Posaconazole

Posaconazole (Noxafil®) is one of the newest azole antifungal drugs introduced. It is approved for use in people but its use in animals has been limited to just a few case reports and pharmacokinetic studies. Posaconazole resembles itraconazole in structure. It is used for invasive fungal infections, including those caused by *Aspergillus* and *Candida*. It is also active against dermatophytes, *Histoplasma capsulatum, Blastomyces dermatitidis, Coccidioides immitis*, and *Cryptococcus neoformans*. Its advantage over other azole drugs is the activity against *Fusarium* and *Mucorales* (formerly called Zygomycetes), such as *Mucor* and *Rhizopus*. It has some chemical properties that are similar to itraconazole and is a substrate for CYP450 enzymes and P-glycoprotein. As with other azoles, the pharmacokinetic/pharmacodynamic parameter that best correlates with clinical success is the plasma concentration AUC/MIC. For treating *Aspergillus* this ratio was above 200; for treating *Candida* the ratio was only 15. If AUC/MIC ratios cannot be monitored, it is suggested to maintain plasma concentrations above (C_{max}) 1.48 μg/ml or an average concentration at least 1.25 μg/ml (Goodwin and Drew, 2008). Additional information has indicated that prophylactic efficacy for invasive fungal infections is optimal when plasma concentrations of posaconazole exceed 0.7 μg/ml 3–5 hours after dosing in a multiple-dose regimen. It is available as an oral suspension containing 40 mg/ml posaconazole

Table 38.5 Topical antifungal products for dogs and cats

Antifungal agent	Product	Indication	Other ingredients
Clotrimazole	Otomax	*Malassezia pachydermatis*	Betamethasone, Gentamicin
Ketoconazole	Keto-Tris Flus	*Malassezia pachydermatis*	Tromethamine (Tris) EDTA
Miconazole	Surolan, and generic	*Otitis externa*	Polymyxin
Nystatin	Dermalone	*Malassezia pachydermatis* (weak activity)	Neomycin
Posaconazole	Posatex	*Malassezia pachydermatis* (with activity against other fungi)	Mometasone, orbifloxacin
Thiabendazole	Tresaderm	*Malassezia pachydermatis*	Dexamethasone, Gentamicin

(Noxafil) and 100 mg delayed-release tablets (Noxafil). The dose in people is 400 mg twice a day with a meal. If not given with a meal, dosage of 200 mg four times a day is recommended. Posaconazole is also one of the ingredients in an approved ear medication for dogs (Table 38.5). This product (Posatex®), contains orbifloxacin, mometasone (glucocorticoid), and 0.1% posaconazole in an otic suspension for the treatment of otitis externa in dogs associated with *Malassezia pachydermatis* and bacteria.

No dose ranges have been established for animals, but in two case reports on its use in cats with successful treatment, a dose of 5 mg/kg orally, every 24 hours was used without ill effects (Wray et al., 2008). Although it is eliminated via UDP-glucuronidation – which may be deficient in cats – the pharmacokinietics of posaconazole in cats resemble dogs, with oral absorption and half-life that are similar to dogs (Mawby et al., 2016b). Based on preliminary pharmacokinetics, the recommended dose for the oral administration of the suspension in cats is 12–15 mg/kg once daily. In dogs, the oral absorption of the suspension is approximately 26% and the half-life is 24 hours. In dogs, there is a food effect on absorption with oral systemic availability of 11% and 27% in fasted and fed dogs, respectively. The delayed-release tablets also offer a good treatment option for dogs. Bioavailability is increased and the half-life is prolonged (42 hours) (Kendall and Papich, 2015). The suggested dosing regimen for the delayed release tablets is 5 mg/kg orally every other day. It is highly protein bound with binding greater than 97% in dogs. In toxicity studies, dogs have tolerated 30 mg/kg/day for 1 year without any clinical signs. However, histologically, some neuronal vacuolation was observed at this dose. It should not be used during pregnancy because of inhibition of steroidogenesis.

Other Antifungal Agents

Terbinafine

Terbinafine (Lamisil®) is a highly fungicidal agent. It is a synthetic drug of the allylamine class. A closely related drug of the same class is naftifine (Naftin®), which is used as a topical cream for dermatophyte infections in people. Terbinafine inhibits squalene epoxidase to decrease synthesis of ergosterol. Fungal cell death results from disruption of the cell membrane (Balfour and Faulds, 1992).

Spectrum of activity: Terbinafine is active against yeasts and a wide range of dermatophytes. It is fungicidal against *Trichophyton* spp., *Microsporum* spp., and some *Aspergillus* spp. (often excluding *A. fumigatus*). It is also active against *Blastomyces dermatitidis, Cryptococcus neoformans, Sporothrix schenckii, Histoplasma capsulatum, Candida*, and *Malassezia* yeast. In people, it was more effective than griseofulvin for treating dermatophytes, with fewer relapses. There may also be some activity against protozoa (e.g., *Toxoplasma*).

Pharmacokinetics: Oral bioavailability in most species is moderate to high, ranging from 31% in cats (Wang et al., 2012) to >46% in the dog to >85% in mice (Jensen, 1989). Absolute bioavailability in horses is unknown; however, the relative bioavailability of terbinafine in horses was only 16% compared to Greyhounds (Williams et al., 2011). Reported half-lives after oral administration of 20–30 mg/kg to cats, dogs, and horses are approximately 8 hours. Maximum concentrations in cats and dogs (3–4 μg/ml) are much higher than those achieved in horses (0.31 μg/ml) (Wang et al., 2012; Williams et al., 2011). Oral absorption of terbinafine has also been studied in Hispaniolan Amazon parrots, with a dose of 60 mg/kg via intragastric gavage resulting in peak plasma concentrations of 0.11–0.67 μg/ml (Evans et al., 2013). In penguins, a dose of 15 mg/kg terbinafine q 24 h is suggested as a potential treatment option for aspergillosis (Bechert et al., 2010).

The lipophilic nature of terbinafine results in high concentrations in tissues such as stratum corneum, hair follicles, sebum-rich skin, and nails. In people, after 12 days of therapy, the concentrations in stratum corneum exceed those in plasma by a factor of 75. Concentrations in skin may be detected in people as early as 24 hours after oral administration, but maximum concentrations are reached at 7 days. Fungicidal concentrations in nails may require 3 weeks of treatment. Concentrations of terbinafine in the hair of cats being treated for dermatophytosis reach approximately 3.62 μg/g after 120 days of treatment with a dose of 30–40 mg/kg. Like itraconazole, terbinafine is highly protein bound, with values reaching >99% in dogs and rabbits (Jensen, 1989).

Clinical use: Terbinafine has some efficacy for the treatment of dermatophytosis in dogs and cats, as well as *Malassezia* dermatitis in dogs. However, there has been conflicting clinical results. For dermatophytes the most common doses are 30–35 mg/kg once daily in dogs; and for cats, approximately 30 mg/kg per day, or one-quarter tablet for small cats (62.5 mg), half tablet for medium size cats (125 mg), and one tablet for large cats (250 mg), all administered once daily. It should be administered for at least 14 days, but may be extended to 60 days. (Moriello, 2004). Results suggest that it is effective for the treatment of *Malassezia* dermatitis in dogs when given at 30 mg/kg PO, or 30 mg/kg two times per week for at least 3 weeks (Berger et al., 2012). However, the results also showed that this treatment produced insufficient resolution and only partial remission even though there was clinical improvement in both groups. In other studies, terbinafine was as effective as itraconazole in treating shelter cats with *Microsporum canis* dermatophytosis when administered in doses of approximately 20–40 mg/kg/day (Moriello et al., 2013). For treatment of *Malassezia* dermatitis, terbinafine (30 mg/kg) was as effective as ketoconazole in reducing yeast counts on the skin (Rosales et al., 2005). Pharmacokinetic studies of 30–35 mg/kg per day in dogs (Williams et al., 2011; Sakai et al., 2011) showed that sufficient concentrations can be maintained for most of the dose interval for susceptible fungi, but clinical studies are needed to confirm efficacy. By comparison, doses in people are much lower at 125 mg twice daily (approximately 1.8 mg/kg q 12 h), with pediatric doses in the range of 4–8 mg/kg, once a day (Jones, 1995).

When combined with surgical excision, terbinafine has been reported to be successful as an adjunct treatment for intestinal pythiosis and *Cryptococcus neoformans* in dogs (Schmiedt et al., 2012; Olsen et al., 2012). It has been safely used in combination with other drugs, including itraconazole and mefenoxam (Hummel et al., 2011) and a synergistic effect against *Pythium* sp. has been shown when combined with itraconazole (Argenta et al., 2008). In both experimental and clinical trials, the average treatment length lasted approximately 60 days. Most treatments in cats are for at least 14 days, but may extend to 60 days. There are currently no published reports of the use of terbinafine in horses, although it has been used clinically at doses of 20–30 mg/kg orally once daily with some success against nasal and guttural pouch fungal masses.

Terbinafine is available as a 1% topical cream (Lamisil®, available over the counter) and 125 and 250 mg tablets. It has also been compounded as a 0.2% solution for ophthalmic use; however, administration did not result in detectable AH or plasma levels following administration to normal equine eyes, suggesting its use may be limited to superficial infections (Clode et al., 2011).

Adverse effects: In dogs treated with 30 mg/kg, serum ALT concentrations were mildly to moderately elevated in 4 of 10 dogs and ALP was increased in 2 of 10 dogs. Owner reported adverse effects include gastrointestinal disturbances and excessive panting (Berger et al., 2012). Gastrointestinal problems, including vomiting, can be common in cats, and facial dermatitis and pruritus has also been reported. This reaction is a problem because it may be confused with an ongoing dermatophyte infection. Pawing at the ground, curling lips, head shaking, anxiety, and circling was noted in one horse after oral administration, but these signs resolved spontaneously within 30 minutes of onset (Williams et al., 2011). In people, there is a rare incidence of severe hepatic failure and death. Terbinafine does not bind to P450 enzymes as do other antifungal drugs; therefore, it does not cause drug interactions or inhibition of steroid synthesis in animals. No teratogenic effects of the drug have been noted in people.

Lufenuron

Lufenuron (Program®), is an orally active inhibitor of chitin synthesis that is commonly used in dogs and cats for control of flea infestations (see Chapter 43 of this book for ectoparasite treatment). It has been evaluated as an antifungal agent, since fungi also have chitin in their outer cell wall. Although there are reports of successful treatment of dermatophytosis in dogs and cats, the success of this treatment has been controversial. If used for dermatophyte treatment, antifungal doses of lufenuron are higher than those recommended for flea control and have ranged from 50 mg/kg to greater than 250 mg/kg. The dose recommended by one group of investigators is 80–100 mg/kg orally, once every 2 weeks until mycological cure (Ben-Ziony and Arzi, 2000). However, endorsement of this use has diminished and dermatologists have disputed the efficacy because of a high incidence of recurrence. It does not have any in vitro effect on *Aspergillus fumigatus* or *Coccidioides immitis*.

It was hypothesized that lufenuron may have a positive effect against dermatological disease not caused by fungi, and an immunomodulatory effect (Zur and Elad, 2006). This is borne out in a study by Mancianti et al. (2009) in which pretreatment with lufenuron followed by treatment with either griseofulvin or topical enilconazole resulted in clinical and mycological cures at a higher rate than expected with any treatment alone.

Topical or local use of lufenuron may be more efficacious. It has been successfully used as a uterine lavage to treat fungal endometritis caused by *Candida* or

Aspergillus spp. in horses (Hess et al., 2002). However, this too is controversial because lufenuron had no in vitro antifungal activity against *Aspergillus* and *Fusarium* sp., pathogens important to horses (Scotty et al., 2005). If administered orally, absorption in horses is poor and an oral dose cannot be recommended. Lufenuron may also be used in water baths for the treatment of aquatic species and amphibians (Wolfe et al., 2001).

Flucytosine

Flucytosine (5-fluorocytosine, 5-FC, Ancobon) is a synthetic antifungal agent available as an oral preparation. Flucytosine must be taken into the fungal cell by cytosine permeate and then converted to the active form, 5-fluorouracil (5-FU), by a fungal cytosine deaminase enzyme. The 5-FU either is incorporated into RNA, disrupting protein synthesis, or is converted to a related compound that inhibits DNA synthesis. Mammalian cells do not have cytosine deaminase, which results in a selective toxicity of this compound; however, conversion to 5-FU may occur by microbes in the gastrointestinal (GI) tract resulting in 5-FU being taken up by mammalian cells, leading to anemia, leukopenia, and thrombocytopenia (Bennett, 1990).

Fungal mutations leading to alterations in the permease or deaminase enzyme activity has led to the development of resistance to flucytosine, both in vitro and during therapy. To decrease emergence of resistance, the use of flucytosine is limited to adjunct therapy with amphotericin B in systemic infections caused by *Candida* or *Cryptococcus neoformans*. Synergy between these two medications, with as much as a fourfold reduction in the MIC, has been demonstrated, and combination therapy has been successful, particularly in the treatment of cryptococcal meningitis (Medoff et al., 1971; Utz et al., 1975; Bennett et al., 1979). One explanation of this synergism involves the membrane-permeabilizing effects of amphotericin B facilitating flucytosine's entrance into the cell cytoplasm (Medoff et al., 1972). This combination of antifungal drugs is more effective than amphotericin B alone in the treatment of cryptococcal meningitis (Bennet et al., 1979; Utz et al., 1975). Advantages of this combination include a reduction in the amphotericin B dose, thereby limiting nephrotoxicity, as well as prevention of mutants to flucytosine (Drouhet and Dupont, 1987). The combination has been administered to treat cryptococcosis in cats at a dose of 250 mg per cat every 8 hours (25–50 mg/kg every 6–8 hours also has been used). This combination has also been suggested for therapy of acute hematogenously disseminated candidiasis (Horn et al., 1985). Although flucytosine is synergistic with amphotericin B, there is no evidence of synergism for azole antifungal drugs and this combination should not be used.

Adverse effects: In one report, the combination of flucytosine and ketoconazole was administered to two cats and liver injury occurred in one cat (Pukay and Dion, 1984). Myelosuppression is also a concern in cats. Cutaneous and mucocutaneous eruptions have been observed with use of flucytosine in dogs and it is not recommended to be used in dogs for this reason.

Sodium or Potassium Iodide

Iodide compounds were one of the first antifungal drugs used and they are still used today in veterinary medicine. They are inexpensive and can be administered orally, which makes them useful for long-term treatment. The mechanism of action of iodide compounds against fungal organisms is largely unknown, but it may involve stimulating the host's immune response or increasing the elimination of the fungi through the skin or hair. Iodide compounds have been used to treat sporotrichosis in dogs, cats, and donkeys, as well as nasal fungal granulomas caused by *Basidiobolus*, *Conidiobolus*, and *Pseudallescheria* spp. in horses (Koehne et al., 1971; Gonzalez Cabo et al., 1989; Irizarry-Rovira et al., 2000; Owens et al., 1985; Zamos et al., 1996; Davis et al., 2000). Ethylenediamine dihydroiodide (EDDI) is used as a nutritional source of iodine in cattle but also has been used to treat fungal granulomatous disease and infections associated with zygomycetes. The antifungal treatment has been questioned because the efficacy is not established.

The iodide compounds are seldom used as a sole therapy, but rather are used as an adjunct to surgical excision, intralesional injection of other antifungals, or systemic antifungal therapy. Toxic effects are possible (iodism) and may occur secondary to excessive iodide levels. Clinical signs attributable to excess iodine include lacrimation, salivation, coughing, anorexia, dry scaly skin, and tachycardia. Abortion and infertility may also be observed; therefore care should be taken when administering this drug to breeding animals. The recommended doses of 20% sodium iodide solution for dogs is 44 mg/kg PO q 24 h, for cats is 22 mg/kg PO q 24 h, and for horses is 125 ml IV q 24 h for 3 days followed by 30 grams PO q 24 h. Treatment is recommended to extend 30 days beyond the resolution of clinical signs. Iodoject IV is labeled for use in cattle for the treatment of actinomycosis and actinobacillosis at 66 mg/kg IV once a week. The use of this product for fungal infections is considered off label.

Topical Antifungal Agents

Enilconazole

Enilconazole (Imaverol®) is also called *imazalil* in some countries. It is an azole antifungal that has excellent activity against dermatophytes and filamentous fungi and it has a residual effect after application. It has been used in some countries for the topical treatment of dermatophyte infections in dogs and horses. For this treatment, a 10% solution is diluted 50 : 1 to form an emulsion. It may be sponged on the animal every 3 or 4 days for four treatments. It may be applied to the premises as well to prevent recurring infections. In an evaluation of topical therapies for treatment of dermatophyte infections in dogs and cats (White-Weithers and Medleau, 1995), enilconazole was more effective than chlorhexidine, povidone iodine, ketoconazole, sodium hypochlorite, and Captan. The safety of enilconazole has been demonstrated in dogs, even at high doses. One study also showed that enilconazole is safe for treatment of dermatophytes in Persian cats (deJaham, 1998). Enilconazole also has been used to treat nasal aspergillosis in dogs. It has a unique vapor effect and, if instilled into the nasal cavity of dogs, will control fungal growth (Sharp et al., 1991; Sharp and Sullivan, 1992). A dose of 10 mg/kg in a volume of 5–10 ml is infused twice a day for 7–14 days. In one study using this protocol (Sharp et al., 1993) 26 of 29 affected dogs became asymptomatic. A similar protocol has been used for the successful medical treatment of guttural pouch mycosis caused by *Aspergillus* spp. in the horse (Davis and Legendre, 1994).

In the United States, enilconazole is available as Clinafarm®-SG or Clinafarm®-EC. Both are approved for use in poultry hatcheries to control *Aspergillus* organisms on facilities and equipment. Clinafarm®-SG comes in a canister used for smoke generation. Clinafarm-EC is available in a 750 ml bottle containing 13.8% (138 mg/ml) enilconazole. The other ingredients listed on the Material Safety Data Sheet are benzyl alcohol and dioctyl sodium sulfosuccinate. It also contains ethoxylated castor oil. The Canadian formulation of Imaverol® contains polysorbate 20 and sorbitan monolaurate as its inert ingredients with 10% enilconazole as the active drug. The Clinafarm-EC formulation is registered for controlling *Aspergillus* organisms in poultry facilities and equipment by making a 1 : 100 dilution and spraying or fogging the area to be treated. Although there are no published toxicology studies on this particular solution in animals, it has been used in a 50 : 1 dilution applied topically to dogs and cats without adverse effects. One study applied 100 ml of a 2 mg/ml formulation of Clinafarm-EC to cats and was judged to be safe (Hnilica et al., 2000). However, monitoring of liver enzymes was recommended by these authors.

Clotrimazole

Clotrimazole (Lotrimin®) is an imidazole antifungal. It is limited to topical use because after oral administration, the metabolism produces undetectable concentrations of the drug in plasma following repeated dosing. In veterinary medicine, it has been used for treatment of nasal aspergillosis in dogs following infusion of a 1% solution over 1 hour through a nasal catheter (Matthews et al., 1998). It has also been infused into the bladder of dogs and cats with fungal candiduria (Toll et al., 2003; Forward et al., 2002). Clotrimazole can be found in combination with gentamicin sulfate and betamethasone valerate as the product Otomax® and other generic forms that are labeled for the treatment of otitis externa caused by *Malassezia pachydermatitis* or susceptible bacteria in dogs (see Table 38.5).

Miconazole

Miconazole (Conofite® cream) is an imidazole antifungal effective against some fungi refractory to amphotericin B. Rapid clearance and poor oral absorption necessitated frequent IV infusions during hospitalization. In addition, the solubilizing agent in the parenteral form induces histamine-related toxic side effects, making its use hazardous. The intravenous formulation is no longer marketed in the United States. Miconazole has a broad spectrum of activity against yeasts and filamentous fungi, although recent evidence suggest that resistance in yeast isolates has been increasing over the last several years (Beltaire et al., 2012). In veterinary medicine, miconazole is used as a 2% cream or 1% spray or lotion for the treatment of dermatophytosis in dogs and cats. It is also commonly compounded as a 1% solution for topical treatment of keratomycosis. The commercially available cream and lotion formulations should not be used in the eye, as they can cause significant irritation. Miconazole can also be found combined with chlorhexidine as a shampoo for the adjunct treatment of dermatophytosis in animals. Antibacterial activity for miconazole has also been demonstrated, and it is a potential treatment for superficial pyoderma caused by methicillin resistant *S. aureus* (Clark et al., 2015), and otitis externa caused by *E. coli* and *P. aeruginosa* (Pietschmann et al., 2013). Synergism in these cases can be achieved by combining miconazole with chlorhexidine for MRSA and polymyxin B for otitis externa. Other products containing antifungal medications are listed in Table 38.5.

Mefenoxam

Mefenoxam (Subdue MAXX®) is an agricultural fungicide that works by blocking ribonucleic acid (RNA) synthesis via inhibition of ribosomal RNA polymerases. It is used to control plant-pathogenic oomycetes. In vitro, mefenoxam has an MIC_{90} of 1 μg/ml against *Pythium insidiosum* isolates (Brown et al., 2008). Results of acute and chronic dosing studies in dogs performed for the EPA certification revealed a no-effect level (NOEL) of 8 mg/kg/day when dosed for 6 months. Although pharmacokinetic data for dogs are not available, rodent studies showed that mefenoxam administered orally to rats at 2 mg/kg produced maximum blood concentrations of 0.48 μg/ml in males and 0.93 μg/ml in females. Extrapolation of these data has led to dosing dogs at 8 mg/kg/day divided into two treatments orally for the therapy of intestinal pythiosis, with some treatment success when combined with itraconazole and terbinafine (Hummel et al., 2011). The drug has been well tolerated in dogs and no persistent clinical, hematological, or biochemical abnormalities have been detected during up to 18 months of administration. It has also been used both systemically and topically in combination therapy for the treatment of canine cutaneous pythiosis and lagenidiosis, as well as topically for the treatment of equine pythiosis.

Natamycin

Natamycin (Natacyn®) is a polyene antifungal with a mechanism of action similar to amphotericin B. It is approved for use in humans as a 5% ophthalmic suspension. Natamycin has excellent activity against yeasts and filamentous fungi, and it is considered the treatment of choice for *Fusarium* keratomycosis in the horse. It is most commonly used in veterinary medicine for treatment of keratomycosis in horses, although it has also been reported as a topical therapy for nasal aspergillosis as well as guttural pouch mycosis and dermatophytosis in this species (Brooks et al., 1998; Greet, 1981, 1987; Oldenkamp, 1979). Its systemic use is prohibited by expense, as well as toxicity.

Nystatin

Nystatin (Mycostatin®) is also a polyene antifungal that is limited to topical use due to systemic toxicity. Nystatin is not absorbed well from the gastrointestinal tract; therefore, it can be given orally as a "topical" treatment for oral and intestinal candidiasis, particularly in exotic animal species (see Table 38.4). In veterinary medicine, it is most commonly used in combination with antibiotics (neomycin, thiostrepton) and antiinflammatory (triamcinolone) drugs in ointments such as Panalog® and other generic formulations as well as otic preparations (Table 38.5).

Antiviral Therapy

Compared to other classes of antimicrobial drugs, the use of antiviral drugs in veterinary medicine has been limited, with all specific antiviral agents used in an extralabel manner. In human medicine, the use of these drugs is much more prominent due to the success of multiple agent protocols to control human immunodeficiency virus (HIV) and associated AIDS, as well as successful single agent therapies for the treatment of herpesvirus infections, influenza, hepatitis C, and other diseases. In veterinary medicine, the most successful use of antiviral agents has primarily been associated with the treatment of herpesvirus infections, with more limited success against retroviruses. Several antiviral drugs are administered systemically, whereas others are only used topically for the treatment of ocular lesions. Consequently, compared to our human medicine counterparts, the consideration of these agents in this book is limited. Readers are encouraged to consult a human pharmacology textbook for more in-depth discussion of mechanism of action and antiviral spectrum of these agents.

There are key differences between the principles of antiviral therapy as compared to antibacterial therapy. Because viruses use host cellular machinery for replication, it is difficult to achieve selective action against viral replication. In this way, many of the most successful of the antiviral drugs act more similarly to the cytotoxic anticancer drugs, by targeting DNA replication. As a consequence the therapeutic indices of antiviral and anticancer drugs may be similarly narrow. For the nucleoside analogs, which are primarily used in the therapy of DNA viruses, there tends to be an inverse relationship between selectivity for viral replication and toxicity to host cells. Another difference between antiviral and antibacterial drugs is that in vitro testing of the antiviral drugs must utilize a cell culture system to allow viral replication. The concentration of drug that inhibits viral growth by 50% is termed the IC_{50}, but this parameter depends upon numerous factors, and is not as robustly associated with efficacy as is the MIC of antibacterial drugs (Hussein et al., 2008a). Antiviral drugs tend to be narrow in spectrum with little cross-activity against multiple viruses. Another feature of antiviral agents that differs from that of antibacterial drugs is that only antiseptic and disinfectant agents can be classified as "virocidal" (antiseptics and disinfectants are discussed in Chapter 31 of this book), so antiviral drugs can only have virostatic actions, limiting replication until the host's immune system eliminates the viral infection. For this reason, antiviral drugs will be inactive against any nonreplicative or latent viral particles. This activity of antiviral drugs only against replicative virus also suggests why timing is critical to successful antiviral therapy, with early therapy resulting in optimal efficacy (Sawtell et al., 2001).

Antiherpesvirus Agents: Nucleoside Analogs

Acyclovir and Valacyclovir

Acyclovir (Zovirax®) is the prototypic nucleoside analog of the purine, deoxyguanosine. In veterinary medicine, the use of acyclovir is restricted to the therapy of herpesviruses. Acyclovir exemplifies selective activity against herpesviruses with little direct effect on host cells at therapeutic concentrations. This selectivity arises from the selective phosphorylation of acyclovir to its active form, acyclovir triphosphate, in infected cells. Acyclovir is monophosphorylated by viral thymidine kinase with 200 times greater efficiency than by the mammalian enzyme, which contributes to its favorable selectivity and high therapeutic index. Sequential phosphorylation by cellular and viral enzymes then form the triphosphate form, which selectively inhibits viral DNA polymerase by competing with deoxyguanosine triphosphate. Acyclo-GTP that is incorporated into viral DNA strands causes termination of elongation. Virally infected cells are 40–100 times more efficient in converting acyclo-GMP to acyclo-GTP than are noninfected cells. Penciclovir and ganciclovir (discussed in Section Penciclovir and Famciclovir and Section Cidofovir, Ganciclovir, and Valganciclovir) have similar mechanisms of action against herpesvirus. Valacyclovir is a prodrug that is itself inactive but is rapidly metabolized to its active form, acyclovir, after oral absorption. The presence of valine allows active transport of valacyclovir into enterocytes, with metabolism by plasma and hepatic esterases to acyclovir.

Spectrum of activity: Whereas herpes simplex viruses (HSV) of people are exquisitely sensitive to acyclovir, few herpesviruses of veterinary importance share this sensitivity. Feline herpesvirus type-1 (FHV-1) is only weakly susceptible to acyclovir with an IC_{50} of 58 μg/ml, more than 100 times higher than that of HSV-1 (Maggs and Clarke, 2004; Gaskell et al., 2007). In contrast, equine herpesvirus type-1 (EHV-1) shares a sensitivity to acyclovir that is similar to HSV, with an IC_{50} of 0.3–3 μg/ml (Wilkins, 2004; Garré et al., 2007b).

Pharmacokinetics: Pharmacokinetic data for acyclovir are available for horses, dogs, and cats, as well as Quaker parakeets, pheasants, and other species (de Miranda et al., 1982, 1981). Oral bioavailability of the drug is high in dogs (>80%), moderate in people (10–30%), but low and variable in horses (<5%). In dogs, oral absorption of acyclovir decreases with escalating doses (Krasny et al., 1981). In horses, the oral bioavailability was undetectable in one study (Wilkins et al., 2005), and only 2.8–4% in other studies (Garré et al., 2007a; Bentz et al., 2006). However, the elimination half-life of acyclovir administered intravenously to horses was reported as 5–53 hours. The high variability is attributed to sampling time and the study conditions (Garré et al., 2007a; Maxwell et al., 2008a). This equine elimination half-life was much longer than in other species (~2 hours in dogs and cats) and suggested the presence of a "deep compartment" in horses. The elimination half-life of acyclovir administered orally to pheasants was similar to that in dogs (3 hours), but was prolonged (15 hours) in box turtles after oral administration of valacyclovir (Rush et al., 2005; Allender et al., 2013). In the species in which mechanisms of clearance have been assessed, acyclovir was primarily eliminated via glomerular secretion (de Miranda et al., 1981, 1982; Krasny et al., 1981). An IV infusion of 10 mg/kg for 1 hour produced effective concentrations in horses for 8 hours and could be administered twice daily for EHV-1 (Wilkins et al., 2005). However, the IV route of administration is seldom used in veterinary medicine (see Section Adverse effects). Because the oral bioavailability of acyclovir is poor in most species, a much better absorbed valine ester prodrug, valacyclovir (Valtrex®), is preferred. In cats and horses, orally administered valacyclovir has much higher bioavailability (more than two times greater in cats; 6–15 times greater in horses) than does acyclovir (Garré et al., 2007a; Owens et al., 1996; Maxwell et al., 2008a).

Clinical use in cats: Clinical signs of herpesvirus infections (feline herpesvirus type-1, FHV-1) in cats prominently consist of upper respiratory disease and ocular lesions (Thomasy et al., 2007; and in review by Gaskell et al., 2007). Since the ocular lesions can progress from conjunctivitis to keratitis, it is the ocular form of the disease that generally warrants antiherpetic therapy. Since acyclovir is poorly absorbed and FHV-1 is relatively insensitive to acyclovir, high doses of valacyclovir were tested in cats in an experimental model of FHV-1. At a dose of 240 mg/kg/day of valacyclovir, nearly six times the maximum labeled dose in people, administration of valacyclovir to cats did not suppress FHV-1 replication but did produce signs of toxicity (Nasisse et al., 1997).

Clinical use in horses: Herpesvirus infections are also important in horses, where EHV-1 is associated with neonatal infections, respiratory disease, abortion, and myeloencephalopathy, depending on the age class of the horse. Oral acyclovir has been used as a treatment for neonatal disease and for equine herpesvirus myeloencephalopathy (EHM) (Murray et al., 1998; Friday et al., 2000). Acyclovir has also been administered to horses with multinodular pulmonary fibrosis (EMPF) attributed to EHV-5, although the sensitivity of EHV-5 to nucleoside analogs has not been determined (Wong et al., 2008). Although it is possible that oral acyclovir may accumulate with multiple doses and result in therapeutic concentrations for highly sensitive isolates associated with a slowly progressive disease such as EMPF, oral acyclovir is too low in horses to justify its use in rapidly progressing herpesvirus infections, such as EHM (Wong et al., 2010). Acyclovir may be more useful as a treatment of neonatal herpesvirus infection. In one herd outbreak of EHV-1 in foals, two of three foals treated with acyclovir survived, whereas the two foals not treated died (Murray et al., 1998). Since this disease is almost universally fatal in foals, acyclovir shows promise as a therapeutict agent.

For the treatment of EHV-1, therapeutic studies have tested the safety and efficacy of valacyclovir, which has been become a more economically feasible option in horses with the availability of generic tablets. Results of experimental trials of the use of valacyclovir against EHV-1 infection have been conflicting. Weanling ponies inoculated with EHV-1 showed neither signs of EHM nor reduction in viremia when valacyclovir was administered at 40 mg/kg every 8 hours for 5–7 days (Garré et al., 2009). However, aged mares appeared to have lower viral loads and be protected from EHM by valacyclovir administration early in the course of disease, at a loading dose of 36 mg/kg, oral, every 8 hours for 2 days, followed by a maintenance dose of 12 mg/kg every 12 hours for 7–14 days (Maxwell et al., 2008b, 2011).

Clinical use in psittacines: In birds, herpesvirus infections can be an important cause of morbidity. Acyclovir has been shown to decrease mortality in psittacine birds with herpesviral infections if the drug is administered prior to the onset of clinical signs (Smith, 1987). Orally administered acyclovir at 80 mg/kg IM every 24 hours after herpesvirus infection in Quaker parakeets was shown to be more effective in preventing death than either low- or high-dose (40 or 250 mg/kg) intramuscular injections. At the highest dose tested, acyclovir toxicity, as seen by local muscular necrosis, was thought to contribute to bird mortality (Norton et al., 1991). In pheasants, a dose of 120 mg/kg of acyclovir administered orally every 12 hours was necessary to maintain concentrations above 1.0 μg/ml, although the safety of this dose rate was not tested (Rush et al., 2005).

Adverse effects: The adverse effects of acyclovir and related drugs have not been well-documented in animals because of their infrequent use. Intravenous administration of acyclovir is seldom used in veterinary medicine due to cost and the increased risk of adverse effects that necessitate IV infusion over 1 hour to diminish the risk of presumed CNS effects (Bentz et al., 2006). Clinical signs of toxicity in cats that received high doses of valacyclovir included nephrotoxicity, as well as the bone marrow suppression that may have been an extension of pharmacological effects, since nucleoside analogs can inhibit cellular DNA replication at high enough concentrations (Nasisse et al., 1997). However, the reported renal tubular necrosis is a known adverse effect associated with high plasma concentrations of acyclovir and is attributed to crystalluria (Sawyer et al., 1988).

Penciclovir and Famciclovir

Penciclovir is administered topically as a 1% cream (Denavir®) for the therapy of herpes labialis in people. When considering the role of nucleoside analogs in veterinary herpesvirus infections, it is worth noting that efficacious topical penciclovir administration in people occurred with early therapy, beginning within 1 hour of lesion appearance, and with application every 2 waking hours. Even then, the main effect of penciclovir administration was to decrease the duration of clinical signs, rather than abolishing such signs. Penciclovir shares a similar mechanism of action as acyclovir, but the intracellular half-life in virus-infected cells is 10–20 times longer (Gill and Wood, 1996). This intracellular accumulation produces an indirect link between plasma penciclovir concentrations and its antiviral effect, and may also represent a therapeutic advantage of penciclovir as compared to acyclovir. Most HSV isolates are similarly sensitive to penciclovir and acyclovir, reflecting their similar mechanisms of action.

Spectrum of activity: As with acyclovir, HSV is quite sensitive to penciclovir, whereas FHV-1 is less so. However, FHV-1 is more sensitive to penciclovir than it is to acyclovir, with an IC_{50} of 3.2–10 μg/ml (Maggs and Clarke, 2004; Hussein et al., 2008b). With a reported IC_{50} of 1.6 μg/ml, EHV-1 may also be more sensitive to penciclovir than it is to acyclovir, although penciclovir sensitivity has been tested in only a single isolate (de la Fuente et al., 1992).

Pharmacokinetics: In rodents and people, the oral absorption of penciclovir is even lower than that of acyclovir. Therefore, an oral diacetate ester prodrug, famciclovir, was developed. It is more lipophilic than the active form, penciclovir, and has much greater oral bioavailability (Gudmundsson and Antman, 2007). After the oral administration of famciclovir to people and rodents, there is extensive metabolism by intestinal esterases and hepatic aldehyde oxidase to produce the active drug, penciclovir (Gudmundsson and Antman, 2007). When 62.5 mg famciclovir (~15 mg/kg) was orally administered to cats, plasma concentrations of penciclovir increased more slowly than in other species, were variable, and suggested saturable absorption (Thomasy et al., 2007). After oral absorption the elimination half-life of penciclovir in cats (3–4 hours) was similar to that of other species. Follow-up studies confirmed the variable and saturable absorption, as bioavailability fell from 12.5% at 40 mg/kg to 7% at 90 mg/kg (Thomasy et al., 2012b). Consequently, dose rates exceeding 40 mg/kg may not produce corresponding increases in plasma penciclovir concentrations. Nonetheless, maximal plasma penciclovir concentrations of 1.3 μg/ml in plasma and of 1.0 μg/ml in tear fluid demonstrated the feasibility of famciclovir administration to cats with ocular signs of FHV-1 infection (Thomasy et al., 2012a). The disposition of penciclovir following the oral administration of 20 mg/kg famciclovir to horses revealed maximal plasma penciclovir concentrations of 2.9 μg/ml, which was higher than in cats (Tsujimura et al., 2010). Interestingly, the elimination half-life of penciclovir after famciclovir administration to horses was prolonged (34 hours) as compared to other species, but similar to the prolonged elimination half-life of acyclovir after valacyclovir administration to horses (Maxwell et al., 2008a; Tsujimura et al., 2010). Despite the theoretical advantages of penciclovir as compared to acyclovir use in horses, studies of the efficacy of penciclovir or famciclovir in protecting horses from signs of EHV-1 or EHV-5 infection are not currently available.

Clinical use: At present, famciclovir is the only systemically administered antiviral agent that is routinely used in cats with ocular or upper respiratory signs of FHV-1 infection. Its efficacy was studied using an oral dose of 90 mg/kg every 8 hours, in cats experimentally inoculated with FHV-1 (Thomasy et al., 2011). In this model, disease score, conjunctivitis, and shedding of FHV-1 were all lower in the cats treated with famciclovir, supporting its clinical utility. However, a lower dose is more commonly used in clinical practice (Malik et al., 2009). It is available as 125, 250, 500 mg tablets, with one-quarter to one 125-mg tablet administered every 8–12 hours most often selected by clinicians. Given that famciclovir absorption profiles are not proportional to dose, it is unknown whether these lower dose rates are as efficacious as was reported experimentally for the high dose rate.

Adverse effects: Whereas one initial case series on the use of famciclovir in cats reported anorexia and polydipsia (Thomasy and Maggs, 2008), a follow-up study in which 90 mg/kg was administered every 8 hours reported no adverse effects (Thomasy et al., 2011).

Cidofovir, Ganciclovir, and Valganciclovir

Cidofovir was the first nucleoside analog approved for clinical use and is still used today as an injectable or ophthalmic implant formulation to treat cytomegalovirus infections in immunocompromised people. Cidofovir is more broadly active against a variety of DNA viruses than are the other nucleoside analogs, as it can be phosphorylated by cellular rather than viral enzymes. Since cidofovir accumulates intracellularly, with a prolonged intracellular elimination half-life, cidofovir can be administered less frequently than the other nucleoside analogs. Cross-resistance between cidofovir and other nucleoside analogs is also unlikely to occur since activation by viral enzymes is unnecessary for the activity of cidofovir. Ganciclovir is also a nucleoside analog but is more potent than acyclovir against herpesviruses, including FHV-1, EHV-1, and human cytomegalovirus (Maggs and Clarke, 2004). Ganciclovir is administered systemically as an injectable formulation for intravenous administration or as an oral tablet. Similar to acyclovir and penciclovir, ganciclovir is poorly absorbed after oral administration, so it has also been formulated as a valine ester prodrug to enhance oral absorption. Although valganciclovir is moderately well absorbed after oral administration to horses, the high cost of this prodrug has precluded further testing in this species (Carmichael et al., 2013). As with the other nucleoside analogs that have been studied in horses, the ganciclovir elimination phase is prolonged as compared to that of other species.

Clinical use: A 0.5% compounded topical formulation of cidofovir is used in cats with herpetic keratitis (Davidson, 2006). Cidofovir ophthalmic solution administered every 12 hours reduced ocular FHV-1 viral load in experimentally inoculated cats, supporting this less frequent dosing interval (Fontenelle et al., 2008). Ganciclovir is a first-line therapeutic agent for cytomegalovirus infections in people, since acyclovir and penciclovir are less potent and less efficacious against these viruses. Ganciclovir is similarly potent when tested in vitro against several herpesviruses of veterinary importance, including the zoonotic, Monkey B virus, endemic to macaques (Brush et al., 2014). Ganciclovir has also been administered intravenously to horses at later stages of infection with EHV-1, when valacyclovir administration may be ineffective (Maxwell et al., 2011).

Adverse effects: Nephrotoxicity is the dose-limiting side effect associated with parenteral cidofovir administration, necessitating coadministration with probenecid to spare the kidneys. Therefore, parenteral cidofovir is not used clinically in veterinary species. However, ophthalmic administration of 0.5% cidofovir appears to be well-tolerated in cats (Fontenelle et al., 2008). Although ganciclovir is more potent than the other antiherpetic guanine analogs, it also has a narrower therapeutic index. Reversible bone marrow suppression, with neutropenia, thrombocytopenia, and anemia, can occur in people that require intravenous doses of ganciclovir, whereas temporary or permanent impairment of fertility as well as teratogenesis occurred in laboratory animals treated with ganciclovir for a prolonged period of time. When administered to mares at a maintenance dose of 2.5 mg/kg every 12 hours for 1 week, side effects were not noted (Maxwell et al., 2011).

Idoxuridine and Trifluridine

Idoxuridine (5-iodo-2′-deoxyuridine, formerly Herplex®, Stoxil®) and trifluridine (5-trifluoromethyl-2′-deoxyuridine, Viroptic®) are thymidine analogs that are only active against DNA viruses, primarily herpesvirus and poxvirus. Like other nucleoside analogs, the compounds are phosphorylated inside the host cell and then incorporated into growing mammalian and viral DNA strands. Only trifluridine is commercially available as a topical ophthalmic preparation for the treatment of herpetic keratitis. The commercial formulation for idoxuridine was withdrawn and is now only available as a compounded solution (0.1%) or ointment (0.5%). Trifluridine (also known as trifluorothymidine) is thought to have higher affinity for viral DNA than mammalian, and so is more potent; however, it also causes the most conjunctival irritation in vivo (Nasisse et al., 1989). Since idoxuridine is better tolerated by cats with herpetic keratitis, it is the more popular choice (Stiles, 1995). As with most other antiherpetic drugs, antiviral effects do not persist once the drug is eliminated from the eye, so the ophthalmic preparations must be applied multiple times per day. Neither drug is administered systemically due to their propensity for toxic side effects.

Cytarabine and Vidarabine

Cytarabine (Ara-C® also known as cytosine arabinoside) and vidarabine (Ara-A®), are nucleoside analogs of cytosine and adenine, respectively. They have in vitro activity against certain DNA viruses, including herpesviruses, poxviruses, vaccinia, rabies, cytomegalovirus, and probably hepatitis B virus. Cellular enzymes convert these compounds to the triphosphate form, which then act as competitive inhibitors of DNA polymerase. As with other antiherpetic drugs that are activated by cellular rather than viral enzymes, cytarbine and vidarabine are not very selective in their activity. As a result, side effects of these drugs have limited the clinical utility of parenterally administered formulations in both humans and veterinary species. Cytarabine is used as an antineoplastic agent in dogs and cats for treatment of leukemia and lymphoma. Its use as an anticancer drug is discussed in more detail in Chapter 44. Cytarabine also is used to treat meningoencephalomyelitis in dogs and is administered either subcutaneously or intravenously (Crook et al., 2013).

Vidarabine is no longer commercially manufactured in the USA, but is occasionally used topically as a compounded 3% ointment in the treatment of herpetic keratitis, although it is less potent against feline herpesviruses than is idoxuridine or trifluridine (Nasisse et al., 1989). The main advantage of vidarabine over the other antiherpetic agents discussed is that viral isolates may not be cross-resistant to both idoxuridine and vidarabine (Eriksson and Oberg, 1979).

Ribavirin

Ribavirin (Virazole®) is a guanosine analog that inhibits the replication of a wide range of RNA and DNA viruses in vitro (Te et al., 2007). In human medicine, ribavirin is primarily used in the therapy of selected RNA viruses, including hepatitis C, respiratory syncytial virus, Lassa fever virus, and influenza A and B. Ribavirin is thought to have multiple sites of action. After being monophosphorylated to ribavirin 5′-monophosphate by adenosine kinase, further phosphorylation to ribavirin 5′-triphosphate predominates intracellularly, and competitively inhibits RNA polymerase and viral replication. Most of the studies investigating the utility of ribavirin against veterinary diseases have been disappointing. Oral ribavirin worsened the condition of cats experimentally infected with calicivirus. Bone marrow suppression, weight loss, increased hepatic enzymes, and icterus were seen (Povey, 1978). These side effects were also seen in healthy cats given the drug (Weiss et al., 1993a); however, they were not seen in dogs when given 60 mg/kg for 2 weeks (Canonico, 1985). Ribavirin was shown to have in vitro antiviral activity against the feline infectious peritonitis (FIP) virus at concentrations of 150 μg/ml (Barlough and Scott, 1989). However, kittens experimentally infected with FIP virus had no significant difference in outcome when compared to those treated with placebo (Weiss et al., 1993b). In fact, similar to the cats infected with calicivirus, the ribavirin-treated FIP kittens had a worsening of clinical signs. In vitro activity against various viral pathogens, including bovine rhinotracheitis and bovine viral diarrhea has also been demonstrated, but there are no reports of the use of this compound in infected animals (Glotov et al., 2004). As is the case with the therapy of hepatitis C infections in people, the efficacy of ribavirin may be enhanced when interferons are coadministered (Carvalho et al., 2014).

Zidovudine, PMEA, and Lamivudine

Zidovudine (Azidothymidine®, AZT®, Retrovir®) is a thymidine analog that was key to the early antiviral success against human immunodeficiency virus (HIV). As such, AZT is a nucleotide analog that selectively inhibits viral reverse transcriptase (nucleoside reverse transcriptase inhibitor, NRTI), preventing viral RNA from making a DNA copy of itself. Like the nucleoside analogs discussed above, AZT is phosphorylated by cellular enzymes to the active triphosphate form. AZT inhibits the viral enzyme with greater affinity (~100 times) than mammalian DNA polymerases, resulting in selective activity and low mammalian toxicity. Adefovir (PMEA) is an acyclic purine nucleoside analog that is used in the therapy of hepatitis and herpesviruses in people, but has been investigated as an antiretroviral drug in cats. Lamivudine is also an older NTRI that has been similarly investigated in cats.

AZT can be given orally or IV in humans. Its oral bioavailability is 60–65%, and peak concentrations are achieved in about 1 hour. AZT is quickly metabolized to the 5′glucuronide, and both the metabolite and parent compound are eliminated in urine with a half-life of approximately 1 hour. The pharmacokinetics of AZT at a dose of 25 mg/kg have been studied in cats following IV, PO, or intragastric (IG) administration through a gastrostomy tube (Zhang et al., 2004a). Absorption following PO administration (95%) was higher than IG administration (70%), but effective plasma concentrations, as determined by the in vitro EC_{50}, were maintained for at least 12 hours following administration by all three routes. As in people, the elimination half-life was short (1.4 hours). No adverse effects, other than hemolysis after

IV injection, were observed in this study; however, only single doses were administered. These same investigators also studied the pharmacokinetics of lamivudine in cats (Zhang et al., 2004b). The pharmacokinetics were similar to those of AZT in cats, but with a high bioavailability, short half-life, and plasma concentrations maintained above the predicted EC_{50} for 12 hours following either IV, PO, or IG dosing of 25 mg/kg.

Clinical use: The use of AZT has been investigated in cats both as a clinical therapeutic and experimentally, using FIV as an animal model of HIV infection. Reverse transcriptase from FIV and HIV-1 viruses was shown to be nearly identical in sensitivity to several antiviral agents, including AZT. In addition, similar concentrations of AZT were required to inhibit replication of these viruses (North et al., 1989). Bovine leukemia virus has also been suggested as a possible animal model for investigation of retroviral infection. AZT inhibition of bovine leukemia virus reverse transcriptase was similar to that of FIV-RT (Reimer et al., 1989).

In experimental FeLV infections, when treated with high doses (20 mg/kg every 8 hours) of AZT within 1 week after virus challenge, kittens were protected from bone marrow infection and viremia. Administration of AZT did not eliminate viremia in kittens if treatment was delayed to 1 and 3 weeks after FeLV inoculation; however, antigen load in the blood was reduced (Tavares et al., 1987). AZT alone, or in combination with interferon or interleukin, was shown to prevent infection in cats challenged with virulent FeLV virus for a period of 6 weeks (Zeidner et al., 1989). However, in cats experimentally inoculated with FIV, even prophylactic use of high doses of AZT did not adequately protect cats from infection (Smyth et al., 1994). PMEA has been studied in the treatment of both FeLV and FIV infections in cats. PMEA was found to inhibit replication of FeLV in vitro and prevented the development of persistent antiginemia and the induction of the immunodeficiency disease in cats inoculated with the virus (Hoover et al., 1991).

AZT has been shown to reduce clinical signs when given to two FIV-positive cats at a dose of 10 mg/kg twice a day subcutaneously for a period of 3 weeks (Egberink et al., 1991). Although it did not eradicate the infection, the authors suggest it is of clinical benefit. Currently, AZT is primarily used to improve clinical signs, such as stomatitis, of cats with FIV and FeLV (Hartmann et al., 1992). In order to reduce dose-limiting side effects, a dose rate of 5 mg/kg PO every 12 hours has been used in symptomatic cats, although efficacy in cats naturally infected with FeLV was not documented (Stuetzer et al., 2013). Seropositive cats with symptoms of opportunistic infection showed improvement of clinical signs during PMEA therapy at 5 mg/kg/day (Egberink et al., 1990). A comparative study on the effects of PMEA and AZT on FIV- or FeLV-positive cats showed that PMEA was superior to AZT in diminishing the clinical signs of disease (Hartmann et al., 1992).

Overall, clinical utility of NRTIs in the therapy of retroviral infections in cats has been disappointing, with only moderate suppression of FeLV and FIV viral loads when therapy is initiated after the infection has become established. Although dose-limiting side effects may partially explain this suboptimal control of retroviral replication, there are also key differences between the much more successfully therapy of HIV in people and the therapy of retroviral diseases in cats. In people, AZT is always combined with other drugs, from a list of 25 antiretroviral drugs in six mechanistic classes, resulting in highly effective antiretroviral therapy (HAART) protocols that are tailored to the needs and responses of individual patients (Panel on Antiretroviral Guidelines for Adults and Adolescents, 2015). Multiple drug protocols are always used to prevent the development of resistance to a single mechanistic class. Monotherapy in cats with AZT or AZT in combination with an immunomodulator, combined with dose-limiting side effects in cats, may limit the ability of antiretroviral therapy to successfully manage FeLV and FIV. Safe and effective combination antiretroviral therapy has not been established in cats. In vitro, lamivudine is synergistic in combination with AZT against FIV, but in chronically infected, experimental cats, the combination did not reduce FIV load and severe side effects were observed (Arai et al., 2002).

Adverse effects: Major toxicities of AZT include anemia and granulocytopenia, which occur in up to 45% of treated human patients (Richman, 1987). Dose escalation studies have demonstrated a dose-dependent and progressive anemia and neutropenia in cats chronically administered >30 mg/kg/day PO divided into three doses for 32–34 days. Marked bone marrow hypercellularity and extramedullary hematopoeisis were observed on postmortem examination (Haschek et al., 1990). In feline patients, Heinz body anemia is the major side effect observed with long-term AZT administration (Hart and Nolte, 1993). When AZT and lamivudine were coadministered to cats at high dose rates, some cats had severe hematological side effects and fevers (Arai et al., 2002). Although PMEA seemed to be more efficacious than AZT in feline patients, the adverse effects (mainly hematological) were more severe with PMEA, limiting its use (Hartmann et al., 1992).

Oseltamivir

Oseltamivir phosphate (Tamiflu®) is an ester prodrug that is converted by hepatic esterases to its active metabolite, oseltamivir carboxylate. In people, only a small percentage of oseltamivir phosphate reaches the systemic circulation, whereas approximately 75% of the oral dose appears in systemic circulation as the active metabolite. Unlike the antiherpetic and antiretroviral drugs discussed above, oseltamivir does not act by directly inhibiting viral replication. Instead, oseltamivir is a competitive inhibitor of the enzyme neuraminidase, which influenza viruses use as part of the process of budding of the replicative viral particles from infected cells. The in vitro efficacy of oseltamivir has been tested against twelve strains of equine influenza A virus (EIV), and most isolates were quite sensitive at IC_{50} concentrations of 4.3–27.5 ng/ml (Yamanaka et al., 2006a). However, one isolate exhibited a much higher IC_{50} of 3785 ng/ml, demonstrating the variability in antiviral susceptibility among influenza strains. Although bioavailability was not determined, when 2 mg/kg of oseltamivir phosphate was administered orally to horses, the resulting plasma concentrations of oseltamivir carboxylate were similar to the plasma concentrations associated with a similar dose in people (Yamanaka et al., 2007). As in people, oseltamivir carboxylate is rapidly eliminated in horses, with a mean elimination phase half-life of 2.5 hours. Given that rapid rate of elimination, the dosing interval of oseltamivir administration in horses may need to be less than 10 hours.

Clinical Use

The efficacy of oseltamivir administered at a dose rate of 5 mg/kg twice daily for 5 days was investigated in a small group of horses experimentally inoculated with EIV (Yamanaka et al., 2006b). Although the small size of the study prevented definitive assessment of drug efficacy, oseltamivir administration did appear to decrease viral shedding, fever, and secondary bacterial pneumonia. Although there is interest in using oseltamivir in recent outbreaks of dogs infected with a canine influenza virus (CIV) that is apparently related to EIV, evidence for its efficacy against CIV is primarily anecdotal. As with most antiviral drugs, the timing of oseltamivir administration is critical to therapeutic outcome in people and must usually be initiated within 48 hours of illness (Rodriguez et al., 2011). Such early initiation of therapy is unlikely in a clinical veterinary setting. Since oseltamivir is an important component in the arsenal against influenza virus infections in people, and resistance to this and other drugs active against influenza can develop rapidly, reserving oseltamivir for human use may be the most prudent approach (Cheng et al., 2010). Indeed, due to concerns regarding pandemic avian influenza virus becoming resistant to oseltamivir (Lee et al., 2011), neuraminidase inhibitors are prohibited by the FDA for extralabel drug use in poultry. Although oseltamivir is not widely used in the therapy of CIV or EIV, it has been used with some regularity in the USA for the therapy of parvovirus infections in dogs. Since parvovirus does not use neuraminidase, the proposed rationale for therapy has instead centered on inhibition of secondary bacterial infections subsequent to parvovirus infections. A clinical trial was performed to test whether addition of oseltamivir at a dose rate of 2 mg/kg PO every 12 hours to a standard protocol improved outcomes in 19 dogs with parvovirus as compared to 16 dogs in the control group (Savigny and Macintire, 2010). Addition of oseltamivir to standard therapy did not affect disease scores, hospitalization time, or mortality, but treated dogs did lose less weight than control dogs and had a less profound decrease in circulating leukocytes. Given that the mechanism of action of oseltamivir does not support efficacy against parvovirus, only a small difference in outcome was associated with oseltamivir therapy, and public health concerns about spread of drug-resistant influenza from dogs to people, oseltamivir use in this setting is difficult to justify.

Adverse Effects

In people, gastrointestinal irritation is the most common side effect associated with oseltamivir administration. Nausea and vomiting have also been observed in dogs during administration of oseltamivir, although this effect may be minimized by dilution of the oseltamivir suspension (Savigny and Macintire, 2010). Side effects were not reported when a similar dose rate of oseltamivir was administered to horses (Yamanaka et al., 2006b).

Amantadine and Rimantadine

Amantadine hydrochloride (1-adamantanamine hydrochloride, Symmetrel®) and rimantadine (Flumadine®) are water-soluble cyclic amines with antiviral activity against a narrow range of RNA viruses, including myxoviruses, paramyxoviruses, togaviruses, and most strains of influenza A virus. Rimantadine has approximately three to four times greater in vitro activity against influenza A than amantadine (Betts, 1991). The mechanism of these two related compounds has been debated. They were thought at one time to prevent viral penetration and uncoating, but this was later refuted (Couch and Six, 1986). Their antiviral activity is now thought to involve inhibition of late-stage assembly of the virus.

Experimentally infected chicks that received amantadine via the drinking water were one-half as likely to die as untreated controls (Obrosova-Serova et al., 1976). However, the use of amantadine in poultry is thought to be responsible for the amantadine resistant influenza strains seen in the Chinese pandemic in 2005 (Ilyushina et al., 2005). Therefore, the FDA now prohibits the extralabel drug use of amantadine and rimantadine in poultry. These two compounds have been investigated for the treatment of influenza in the horse. In vitro testing suggests that amantadine suppresses viral replication at concentrations of 300 ng/ml while rimantadine is more potent and has activity at concentrations as low as 30 ng/ml (Rees et al., 1997, 1999). Amantadine caused serious adverse effects, including fatal seizures, in horses at doses of 10–15 mg/kg IV. Absorption of the drug following oral administration was highly variable between individual horses; therefore, a dose could not be recommended (Rees et al., 1997). Rimantadine shows greater promise as an antiviral drug in horses. A multiple dose study examining the effects of oral rimantadine at a dose of 30 mg/kg q 12 h showed adequate absorption of the drug with plasma concentrations maintained above the estimated effective concentration (30 ng/ml) throughout the dosing interval. No side effects were reported. In challenge studies using influenza virus A2, prophylactic rimantadine administration caused a significant decrease in rectal temperature and lung sounds (Rees et al., 1999). However, both amantadine and rimantadine have fallen out of favor for the treatment of influenza in people. Although amantadine and rimantadine were originally introduced as antiviral agents, their poor efficacy in human influenza patients and the likelihood of adverse effects, such as gastrointestinal irritation and central nervous system effects, has resulted in recommendations against their use for influenza virus (Jefferson et al., 2006).

Treatment of Pain

An additional use of amantadine is for treatment of pain syndromes in animals. The proposed mechanism of action for amantadine is via inhibiting the neurotransmitter *N*-methyl-ᴅ-aspartate (NMDA) (Pozzi et al., 2006). NMDA produces central sensitization and pain in animals. Blocking of the NMDA central receptor by amantadine has been associated with relief of pain syndromes. Amantadine is well absorbed orally in practically all animals, but the precise duration of action and dosing regimens have not been fully investigated in animals. In one study, amantadine was administered for 21 days, in combination with meloxicam, for the alleviation of refractory osteoarthritis pain in dogs (Lascelles et al., 2008). At a dose of 3–5 mg/kg orally, once daily, with meloxicam, dogs responded better than if administered meloxicam alone. Other doses that have been cited for pain are 2–10 mg/kg orally every 8–12 hours in dogs and 2 mg/kg orally every 24 hours in cats (Pozzi et al., 2006).

Interferon

A complete discussion of the biochemistry, physiology, and immunological function of interferon-$\alpha_{2a,2b}$ (Roferon-A, Intron A) is beyond the scope of this chapter. Interferons are polypeptide molecules produced by certain mammalian cells in response to viral infections as well as other stimuli. They are potent cytokines that possess antiviral, immunomodulating, and anticancer properties (Pestka et al., 1987). Interferons are divided into three types: type I, which includes IFN-α, IFN-β, and IFN-ω, type II, composed of only IFN-γ, and type III, which are the IFN-λs (Hoffmann et al., 2015). The antiviral activities of interferons are indirect. They induce a variety of antiviral mechanisms via enhancement of IFN responsive gene promoters within the host cells. An interesting property of IFNs is that once they induce an antiviral state in one cell, that cell can then transfer this antiviral activity to other cells through cell-to-cell contact without requiring additional IFN. This has been demonstrated in vivo using IFN-α stimulated lymphocytes. This results in an amplification of the antiviral effect, which may explain why IFNs are efficacious despite low to undetectable plasma levels (Stanton et al., 1989). Some interferons are highly conserved between species and therefore their actions are not species specific. In people, multiple interferons have been used for treatment of HIV and cancer-associated diseases.

Interferons have been administered parenterally, intranasally, and orally. Parenteral and intranasal administration lead to an increased risk of adverse effects, including formation of neutralizing antibodies to IFN, as well as clinical signs of hyperthermia, anorexia, and malaise (Roney et al., 1985). Being peptides, interferons are inactivated by the digestive enzymes in the gastrointestinal tract, yet orally administered IFN has been shown to be an effective treatment for viral diseases in a number of species (Cummins et al., 1999). The probable mechanism for this is uptake of IFN by the oropharyngeal-associated lymphoid tissue, which sensitizes the lymphocytes in these organs. The lymphocytes are then released into the circulation where they can confer antiviral activity to cells at the site of infection (Bocci, 1991).

Alpha Interferon

In vitro studies have demonstrated that FHV-1 is susceptible to feline interferon (IFN-ω) or human IFN-α (Siebeck et al., 2006). Interferons and antiviral drugs, such as acyclovir, can act synergistically to inhibit viral replication (Weiss, 1989). The human formulation of IFN-α is available as an injectable formulation, but it has been administered in an extralabel manner by both oral and parenteral routes to veterinary species. Surprisingly, a dose as low as 0.5 U administered PO to cats appears to exert antiviral activity (Cummins et al., 1988). However, oral administration of IFN-α to horses at doses of 0.22 to 2.2 U/kg just before and after inoculation with EHV-1 failed to produce a protective response (Seahorn et al., 1990). Most of the use of IFN-α has been

Table 38.6 Clinical and experimental uses of interferon in veterinary species

Species	Disease treated	IFN Subtype	Dose and route of administration	Results	Reference
Feline	FeLV	Human IFN-α	1.6×10^4–1.6×10^6 U/kg with or without AZT at 10 mg/kg q 8 h	IFN administration decreased p27 antigen, but cats became refractory in 3–7 weeks, whereas AZT had no effect	Zeidner et al., 1990
	FIP (coronavirus)	Recombinant feline IFN-γ	1 million U/kg SC EOD until remission followed by weekly injections of the same dose	Produce complete remission (>2 years) in 4/12 cats, and partial remission (2–5 months) in 4/12 cats; only cats with the effusive form of FIP responded.	Ishida et al., 2004
	FeLV or FeLV/FIV coinfection	Recombinant feline IFN-ω	1 million U/kg SC q24h for 5 consecutive days in 3 series (day 0, 14, and 60)	Placebo controlled study; treated cats had significantly lower clinical scores in the first 4 months and significantly lower mortality rates at 9 and 12 months.	de Mari et al., 2004
	FeLV	Human IFN-α with or without *Staphylococcus* Protein A (SPA)	30 U/cat PO once daily on weeks 1,3,5,7, and 9	Placebo controlled study; no significant improvements seen in animals treated with IFN compared to controls; mild improvement noted based on owner perception in cats treated with SPA alone.	McCaw et al., 2001
	FIV	Natural human IFN-α	10 IU/kg PO q24h on alternating weeks for 6 months	Treated cats had a significant improvement in clinical signs and a longer survival than controls; no correlation with plasma viremia or viral load in leukocytes was noted.	Pedretti et al., 2005
	FeLV, FIV	Recombinant feline IFN-ω	1 million U/kg SC q24h for 5 consecutive days in 3 series (day 0, 14, and 60)	Cats treated with IFN had better improvement in clinical scores as compared with control cats, but proviral load and viremia were not affected.	Domenech et al., 2011
	FeLV	Human IFN-α	$1\text{x}10^5$ U/kg with or without AZT at 5 mg/kg q 12 h	No significant differences between placebo control and drug treatment groups in p27 antigen.	Stuetzer et al., 2013
Canine	Parvovirus (CPV-2)	Recombinant feline IFN-ω	1 million U/kg IV q24h for 3 consecutive days starting 4 days after viral inoculation	Placebo controlled study; treatment significantly reduced the severity of the enteritis within 12 hours of administration of the first dose; all dogs received standard supportive therapy.	Ishiwata et al., 1998
	Parvovirus	Recombinant feline IFN-ω	2.5 million U/kg IV q24h for 3 consecutive days	Multicentric, double-blind, placebo controlled field trial; treated dogs showed a significant improvement in clinical signs; mortality rate was 7% in treated dogs and 29% in controls; all dogs received standard supportive therapy.	de Mari et al., 2003

(*continued*)

Table 38.6 (*Continued*)

Species	Disease treated	IFN Subtype	Dose and route of administration	Results	Reference
Bovine	Vaccinia (Smallpox)	Natural human IFN-α2	10 million U/kg IM q24h starting 24 hours prior to viral inoculation	Placebo controlled study; complete protection was obtained at this dose.	Werenne et al., 1985
	IBR	Recombinant human IFN-α	0.05–5 U/kg PO q24h for 4 days starting 48 hours prior to intranasal viral inoculation	Placebo controlled study; the 0.05 and 0.5 U/kg groups showed significant improvement in mean rectal temperature and duration of antibiotic therapy; the 0.05 U/kg group also had significantly better weight gain.	Cummins et al., 1993
	IBR	Recombinant bovine IFN-α1	10 mg/animal intranasally 48 hours prior to viral inoculation	Significant reduction in morbidity and mortality in treated animals was noted, although treatment did not prevent clinical signs or affect viral shedding.	Babiuk et al., 1987
	BLV	Recombinant bovine IFN-τ	10^5–10^6 U/kg SC 3x/week for 3–4 weeks	Reduced titers of BLV in IFN treated cattle	Basu et al., 2006
	BVDV	Recombinant bovine IFN-τ	10^5–10^6 U/kg SC 5x/week for 2 weeks	Serum titers to BVDV decreased slightly in the high dose group, but only during the administration period	Kohara et al., 2012
Swine	FMDV	Adenovirus expressing porcine IFN-α or IFN-γ, alone and in combination	Low and high doses of each cytokine	Swine with the combination of the two IFNs were completely protected from challenge infection, in contrast to control animals	Moraes et al., 2007
Equine	EHV-1	Recombinant human IFN-α2a	0.22 or 2.2 U/kg PO 48 and 24 hours prior to viral inoculation and 24 hours after inoculation	No significant effects on clinical disease or duration of viral shedding were noted.	Seahorn et al., 1990
	Inflammatory airway disease	Recombinant and natural human IFN-α	90 U/horse (recombinant) or 50 U/horse (natural) PO q24h for 5 days	Placebo controlled study; all horses had a significant decrease in cough and nasal discharge; significantly fewer horses treated with either IFN product had a relapse after 4 weeks; viral etiology in this disease not proven.	Moore et al., 2004
	Shipping fever	Human IFN-α	1.25 g/head PO q24 h for 4 days	Placebo controlled study; elevations in white blood cells, fibrinogen, serum amyloid A were partially mitigated by IFN administration.	Akai et al., 2008

BLV, bovine leukemia virus; BVDV, bovine viral diarrhea virus; CPV, canine parvovirus; EHV, equine herpesvirus; FeLV, feline leukemia virus; FIP, feline infectious peritonitis; FMDV, foot-and-mouth disease virus; IFN, interferon; IBR, infectious bovine rhinotracheitis.

in experimentally infected animals or anecdotal, with few clinical trials to demonstrate efficacy for treating FHV-1 infections (Gaskell et al., 2007).

Omega Interferon

Omega interferons are licensed for use in the treatment of viral disease of dogs and cats in Europe, Japan, Australia, New Zealand, and Mexico. Omega interferon of feline origin, produced by genetic engineering, is a type 1 interferon closely related to α-interferon (Yang et al., 2007). This IFN-ω binds to the same receptors as IFN-α and IFN-β. Recombinant IFN-ω contained in Virbagen Omega is produced by silkworms previously inoculated with interferon-recombinant baculovirus, resulting in the synthesis of pure interferon.

After injection, IFN-ω has a half-life of 1.4 hours in dogs and 1.7 hours in cats. The IFN-ω is rapidly cleared by the kidneys and is not widely distributed. Instead, it is bound to receptors in cells infected by virus. Interferon has been used to stimulate immune cells in dogs with parvovirus and in cats with feline retrovirus (FeLV and FIV), which are the labeled indications for Virbagen Omega.

Doses and indications for animals have primarily been based on extrapolation of human recommendations, experimental studies in animals, or specific studies in cats and dogs with viral infections. The formulation used in veterinary medicine is available in 5 and 10 million units/vial, which is reconstituted before use. The suggested dose in dogs is 2.5 million units (MU)/kg IV once daily for 3 consecutive days. The dose in cats is 1.0 MU/kg SC for 5 consecutive days. Three separate 5-day treatments must be performed at day 0, day 14, and day 60. A summary of some of the reports of interferon use in natural and experimental viral disease of different species is presented in Table 38.6.

Adverse reactions: In people, injections of IFN-α have been associated with influenza-like symptoms. Other effects also have been reported in people, such as bone marrow suppression. In animals, interferon has been generally well tolerated, but may induce vomiting and nausea. In some animals it may induce hyperthermia 3–6 hours after injection. In cats, it may produce soft feces to mild diarrhea. A slight decrease in white blood cells, platelets, and red blood cells, and rise in the concentration of alanine aminotransferase may be observed. These parameters usually return to normal in the week following the last injection. In cats, it may induce transient fatigue during the treatment. Do not vaccinate dogs or cats receiving interferon.

L-Lysine

L-lysine is an essential amino acid that blocks the availability of arginine, which is necessary for the replication of herpesviruses. In vitro testing has demonstrated an inhibitory effect of L-lysine on FHV-1 replication in the presence of arginine (Maggs et al., 2000). Additionally, a dose of 400 mg per day, given in food, reduced viral shedding following the stress of changes in housing and husbandry (Maggs et al., 2003). This beneficial effect was blocked when the cats were given methylprednisolone to induce viral shedding. When given prophylactically at a dose of 500 mg 6 hours prior to viral challenge, and then continued at 500 mg PO q 12 h, it decreased the severity of the ocular lesions compared to controls; however, it had no effect on virus isolation (Stiles et al., 2002). Despite the promising results cited above, clinical results have been disappointing. A clinical study in shelter population cats did not show a benefit of treatment (Rees and Lubinski, 2008). When 144 treated shelter cats were compared to 147 controls, there was no difference in prevention of upper respiratory infection or conjunctivitis when cats were supplemented with 250 or 500 mg daily oral L-lysine. There was actually an increase in severity of some infections. For these reasons, the use of L-lysine to treat FHV-1 in shelter cats is not recommended until reevaluation is available.

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Section 10

Chemotherapy of Parasitic Diseases

39

Antinematodal Drugs

Carlos E. Lanusse, Juan M. Sallovitz, Sergio F. Sanchez Bruni, and Luis I. Alvarez

The economic importance of helminth infections in livestock has long been recognized and it is probably for this reason that the most important advances in the chemotherapy of helminthiasis have come from the animal health area (Horton, 1990). Anthelmintics are used in all animal species and man. A significant part of the economic impact of parasitism in animal production is represented by the investment in control measures. Although alternative methods have been developed, chemically based treatments are the most important tool to control parasitism. A more complete understanding of the pharmacological properties of existing antiparasitic drugs should assist with more efficient parasite control both in livestock and companion animals. However, the investment in control measures does not always result in the expected therapeutic success. Among factors responsible for that therapeutic failure are: (i) inadequate integration between management strategies and chemotherapy; (ii) incorrect use of anthelmintic drugs due to insufficient knowledge of their pharmacological features; and (iii) insufficient understanding of the relationship between pharmacological properties and several host-related factors that could lead to modifications of the pharmacokinetic behavior and to a decreased antiparasite efficacy of the chosen drug. In addition, the availability of many compounds with a common mode of action and the indiscriminate use of these drugs have accounted for the widespread development of drug resistance, mainly in parasites of sheep and goats, but also in parasites of pigs, horses, and cattle. This chapter covers the information available for the different specific antinematodal drug families used in veterinary therapeutics. However, special consideration is given to the description of the large body of pharmacological knowledge generated for the benzimidazole compounds, the most intensively studied anthelmintic chemical group together with the endectocide macrocyclic lactones (see Chapter 41).

Benzimidazoles and Probenzimidazoles

Benzimidazoles (BZD) and pro-benzimidazoles (pro-BZD) anthelmintics are widely used in veterinary and human medicine. The pro-BZD are inactive prodrugs metabolically converted into anthelmintically active BZD molecules in the host. The remarkable overall safety of BZD compounds has been a major factor in their successful worldwide use over four decades. BZD were introduced into the animal health market primarily for the control of gastrointestinal (GI) nematodes, not only for use in livestock animals (cattle, sheep, goats, swine, and poultry), but also for horses, dogs, and cats. The use of BZD compounds quickly became widespread because they offered major advantages over previously available drugs in terms of spectrum, efficacy against immature stages, and safety for the host animal.

Chemistry

Since the discovery of thiabendazole in 1961 (Brown et al., 1964), several thousand BZD molecules have been synthesized and screened for anthelmintic activity. However, no more than 20 of them have been commercially developed for use in domestic animals and man, either as BZDs or pro-BZDs. The BZD structure is a bicyclic ring system in which benzene has been fused to the 4- and 5- positions of the heterocycle (imidazole) (Figure 39.1). Most of the BZD compounds are white crystalline powders, with fairly high melting point and insoluble or slightly soluble in water. Their aqueous solubility is markedly higher at low acidic pH values, with the stomach/abomasum being the appropriate site for the dissolution of BZD drug particles after oral treatment. The BZD compounds can be grouped as follows:

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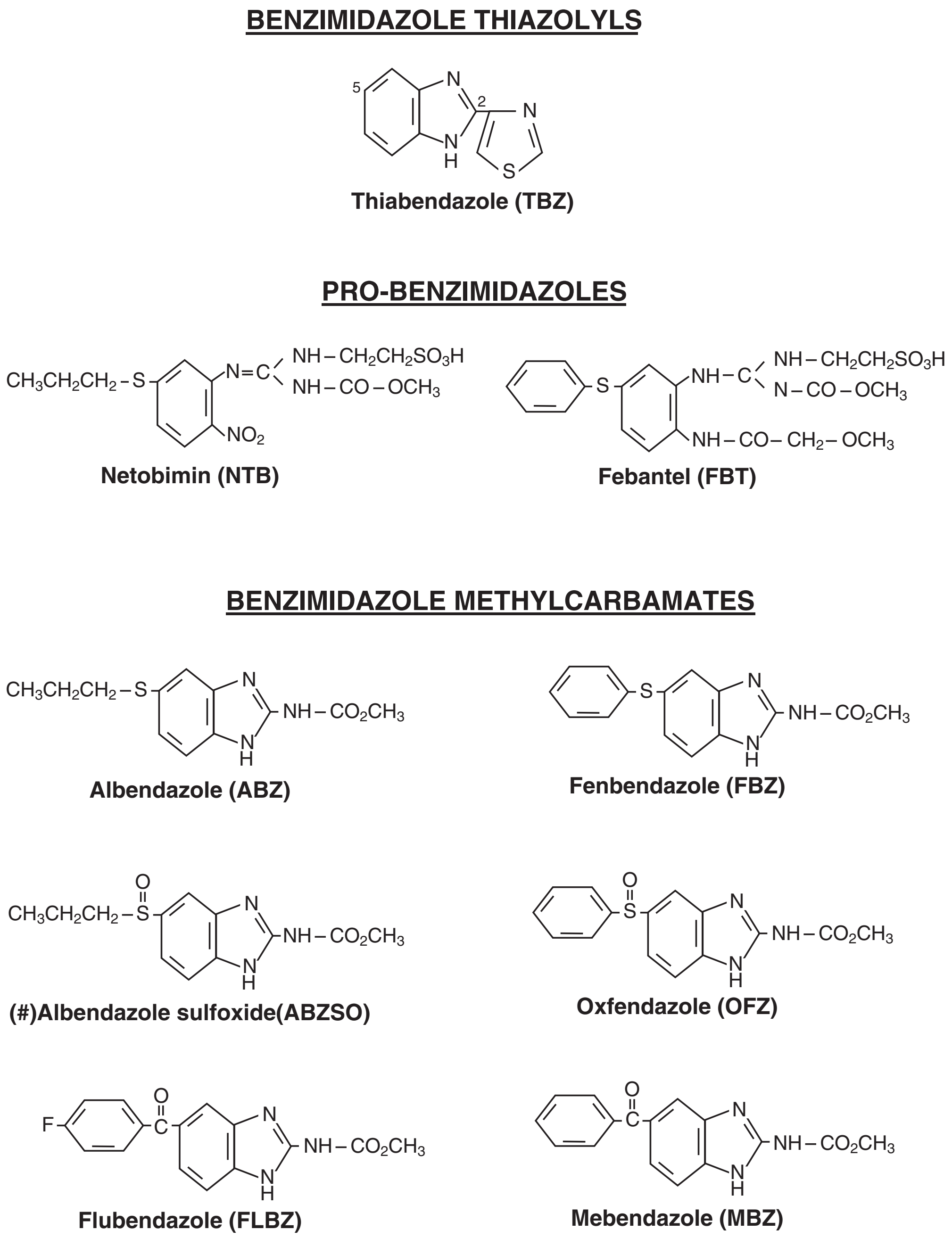


Figure 39.1 Chemical structures of the main benzimidazole (BZD) anthelmintics used in veterinary medicine. The positions 2- and 5- (main substitution sites) are shown in the thiabendazole structure. (#) ABZSO is also known as ricobendazole (RBZ). Triclabendazole (a halogenated flukicidal BZD) is not included here (see Chapter 40).

1) *BZD thiazolyls*: thiabendazole, cambendazole;
2) *BZD methylcarbamates*: parbendazole, mebendazole, flubendazole, oxibendazole, luxabendazole, albendazole, albendazole sulfoxide, also known as ricobendazole, fenbendazole, oxfendazole;
3) Halogenated BZD thiols: triclabendazole;
4) *pro-BZD*: thiophanate, febantel, netobimin.

Different modifications at positions 2- and 5- of the BZD ring system (see Figure 39.1) have provided the most anthelmintically active drugs, especially, with the discovery of sulfur-containing methylcarbamate derivatives such as albendazole and fenbendazole, which exhibit high efficacy against lungworms and inhibit larval stages of most GI nematodes. The BZD methylcarbamates are the most used BZD compounds. Albendazole, fenbendazole and their sulfoxide derivatives (albendazole sulfoxide and oxfendazole, respectively) (ruminants), fenbendazole and oxfendazole (horses), febantel, fenbendazole, and mebendazole (companion animals), fenbendazole and flubendazole (poultry and pigs) are currently among the most extensively used BZD methylcarbamate anthelmintics in veterinary medicine.

Pharmacodynamics: Mode of Action

Microtubules are hollow tubular organelles that exist in a dynamic equilibrium with tubulin, the microtubule subunit. Tubulin is a dimeric protein comprised of α- and β-subunits. The BZD and pro-BZD pharmacological activity is based on the binding to parasite β-tubulin, which produces subsequent disruption of the tubulin–microtubule dynamic equilibrium (see Lacey, 1990 for detailed information). Competitive binding experiments using tubulin from mammalian, invertebrate, or fungal cells indicate that BZD compounds bind within the colchicine (a well recognized microtubule inhibitor) binding domain on tubulin. Thus, all the functions ascribed to microtubules at the cellular level are altered (cell division, maintenance of cell shape, cell motility, cell secretion, nutrient absorption, and intracellular transport) (Lacey, 1988). Microtubules are found in animals, plants, and fungi cells. However, the rate constant for the dissociation of BZD from parasite tubulin is much lower than the rate constant for the dissociation from mammalian tubulin. These differences in dissociation rates between BZD and tubulin in host and parasites may explain the selective toxicity of BZD compounds to parasites and its wide safety margin in the mammalian host. The microtubule loss observed at tegumental and intestinal level in cestodes and nematodes after BZD treatment is followed by loss of transport of secretory vesicles and decreased glucose uptake. A prolonged storage of secretory material within the cells is followed by cell disintegration. Cell autolysis requires a period of 15–24 hours posttreatment. Additionally, the inhibition of secretion of nematode acetylcholinesterase and inhibition of some enzymatic activities (such as fumarate reductase, malate dehydrogenase, phosphoenol pyruvate reductase, and succinate dehydrogenase) have been associated with the BZD anthelmintic action. However, all these effects may be related to the primary underlying BZD mechanism: the disruption of the tubulin–microtubules dynamic equilibrium. The effects of structural modifications to BZD-like molecules on microtubule inhibitory activity have been intensively investigated (reviewed by Lacey, 1988). It has been postulated that the presence of a carbamate group in the 2- position is essential for potent microtubule inhibitory activity. Additionally, regardless of the size of the substituent in the 5- position, the pharmacological effect heavvily depends on the nature of the molecule adjacent to the BZD ring system. Different studies suggest that not only the chemical substitution in the position 5- of the BZD ring, but also its conformational arrangement, are relevant in the access of the drug to the active site and in the resultant anthelmintic activity.

Pharmacokinetic Behavior

The anthelmintic activity of BZD compounds not only depend on its binding to β-tubulin, but also on their ability to reach high and sustained concentrations at the site of parasite location, which allow the delivery of effective concentrations of the compound at the receptor within the parasite cells, in sufficient time, to cause the therapeutic effect (Thompson et al., 1993). As a chemical class, the BZD methylcarbamates have only limited water solubility and small differences in drug solubility may have a major influence on their absorption and resultant pharmacokinetic behavior. The lack of water solubility is an important limitation for the formulation of BZD compounds, which mainly allows their preparation as suspensions, pastes, or granules for oral or intraruminal administration. Poor/erratic GI absorption is a common inconvenience for the systemic availability of enterally administered BZD suspensions in most species. The mucous surface in the GI tract behaves as a lipid barrier for the absorption of active substances, so that absorption depends on lipid solubility and degree of ionization at GI pH levels. However, drug particles must dissolve in the enteric fluids to facilitate absorption of the BZD molecule through the GI mucosa. A drug that does not dissolve in the GI contents, passes down and is excreted in the feces without exerting its action. The BZD methylcarbamates show only limited GI absorption due to their poor solubility in water. Their dissolution rate, passage along the GI tract, and absorption into the systemic circulation, are markedly slower than those observed for the more hydrosoluble BZD thiazolyls (TBZ, thiabendazole). Such a phenomenon also results in extended residence times for the active metabolites of the BZD methylcarbamates compared to those of TBZ. This differential pharmacokinetic behavior accounts, in part, for the greater anthelmintic potency of fenbendazole and albendazole compared with thiabendazole.

Pharmacokinetics and Metabolism in Ruminant Species

Absorption

The complexity of the ruminant digestive tract in comparison to that of the monogastric animal creates unique problems and opportunities related to the absorption of drugs administered orally. The rumen may substantially influence the absorption pattern and the resultant pharmacokinetics and antiparasite activity of enterally delivered BZD anthelmintics. The rumen accounts for about 20% of the animal’s volume and never empties. When a BZD suspension is deposited in the rumen, solid particles mix and distribute through the digesta volume.

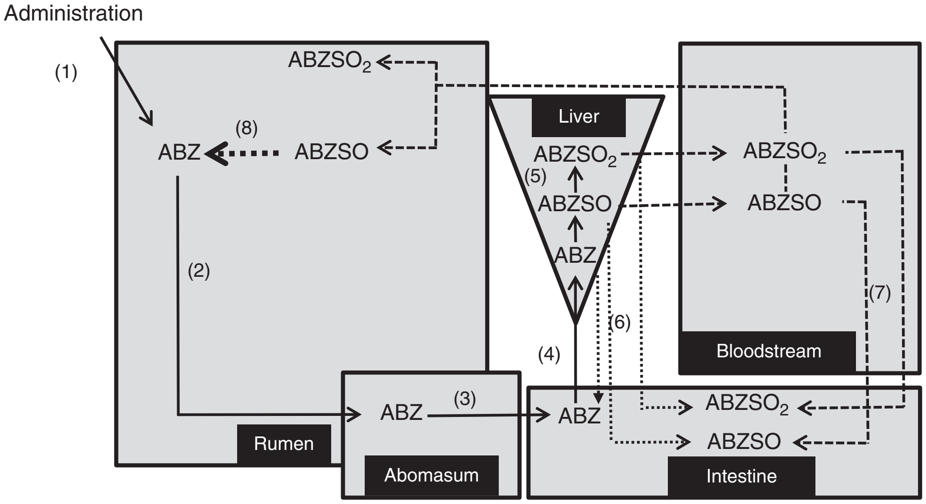


Figure 39.2 Schematic representation of the main pharmacokinetic features taking place after oral/intraruminal administration of benzimidazole anthelmintics in ruminants. See the text for further explanation. Albendazole (ABZ) is used as a model drug in the scheme.
(1) Route of administration: only oral or intraruminal routes can be used for the sulfide compounds (ABZ, FBZ).
(2) Rumen: marked drug dilution. The rumen's reservoir effect is by drug adsorption to particulate digesta.
(3) Abomasum: the acidic pH facilitates drug particles dissolution and strong "ionic trapping" of the drug/metabolite at the abomasum.
(4) Small intestine: main drug absorption site.
(5) Liver: drug oxidative metabolism.
(6) Biliary system: drug excretion (unconjugated and conjugated metabolites), enterohepatic recycling.
(7) Bloodstream: metabolites distribution to different organs/tissues, including active secretion to gastrointestinal tract.
(8) Rumen: sulforeductive biotransformation by ruminal microflora.
ABZ, albendazole parent compound; ABZSO, albendazole sulfoxide; ABZSO$_2$, albendazole sulfone.

Shortly after administration, BZD compounds are almost completely associated (adsorbed) to the digesta particulate material. The drug reaches an equilibrium between the particulate and fluid portions of the digesta. The drug dissolved in the GI fluid is available for absorption and/or for diffusion through the external surface of the helminth parasite located at the GI lumen. The adsorption of BZD particles to digesta solid content, the slow mixing and long digesta residence time, and the large rumen volume assists absorption by delaying the rate of passage of drug down the GI tract (Hennessy, 1993). The rumen acts as a drug reservoir by slowing the digesta transit time throughout the abomasum, which results in improved systemic availability of BZD compounds as a consequence of a greater dissolution of drug particles in the acid pH of the abomasum (Lanusse and Prichard, 1993a). The plasma levels of the parent sulfides (i.e., albendazole, fenbendazole) and/or its active sulfoxide metabolites (albendazole sulfoxide, oxfendazole) reflect the amount of dissolved drug at the GI level (Figure 39.2). Since the main mechanism of drug entry to nematode parasites located in the GI lumen is drug diffusion through its external surface, the higher the concentration of solubilized drug in the GI fluid, the greater the anthelmintic activity. The influence of the rumen on BZD absorption is evidenced by the higher and more sustained concentration of fenbendazole, oxfendazole, albendazole, and their metabolites recovered in the bloodstream after oral/intraruminal treatments compared to intraabomasal administration of the same compounds in sheep (Prichard et al., 1978; Marriner and Bogan, 1981).

A lack of proportionality in the relationship between albendazole dose rates and drug systemic exposure has been described in sheep, where area-under-the-curve (AUC) and maximum peak concentration (C_{max}) values for albendazole sulfoxide increased more than expected from the increase in the dose. The enhanced systemic exposure achieved after albendazole treatments at the highest dose rates correlated with significant increment in drug efficacy against a resistant *Haemonchus contortus* strain (Alvarez et al., 2012).

Metabolism and Elimination

BZD and pro-BZD anthelmintics are extensively metabolized in all mammalian species studied. As a common pattern among different BZD compounds, the parent drug is short lived and metabolic products predominate in plasma and all tissues and excreta of the host, as well as in parasites recovered from BZD-treated animals (Fetterer and Rew, 1984; Alvarez et al., 1999, 2000). The primary metabolites usually are products of oxidative and hydrolytic processes and are all more polar and water soluble than the parent drug. In addition, phase

II conjugative reactions are highly important in the detoxification of BZD-derived products. The oxidized and hydrolyzed metabolites are conjugated with glucuronide and/or sulfate to increase their polarities, which facilitates urinary or biliary excretion. Since BZD anthelmintics are mainly administered by the oral route, "first-pass" metabolism is relevant in the kinetic behavior of these compounds. Intestinal, liver, and lung metabolism have been implicated in this phenomenon in most animal species. Additionally, GI metabolism is an important concern in ruminant species.

Netobimin (pro-BZD) is an anthelmintically inactive nitrophenylguanidine prodrug. Netobimin is nitroreduced and cyclized into albendazole in a microflora-mediated reductive reaction in the GI tract (Lanusse et al., 1993a). The nitro-reduction and cyclization of netobimin into albendazole in the host are crucial for the pharmacokinetic profile of its active metabolites and resultant anthelmintic activity. Both formulation and route of administration may dramatically affect the rate of netobimin conversion and the bioavailability and disposition of its main plasma metabolites, albendazole sulfoxide and albendazole sulfone. Sheep and cattle ruminal and ileal fluids can convert netobimin into albendazole under in vitro conditions (Lanusse et al., 1992b). However, the subcutaneous administration of netobimin resulted in lower plasma concentrations of active metabolites compared to those measured after oral administration of the prodrug in sheep and cattle (Lanusse et al., 1990). In conclusion, oral administration results in an improved pharmacokinetic profile of netobimin metabolites, which accounts for some advantageous anthelmintic efficacy after the oral/intraruminal compared to the parenteral treatment.

Febantel is a phenylguanidine prodrug, which is hydrolyzed by removal of a methoxyacetyl group and then cyclized to fenbendazole. Fenbendazole is then converted to oxfendazole and fenbendazole sulfone in subsequent oxidative metabolic steps. Following febantel administration, in both sheep and cattle, the parent drug is not found in plasma, or is detected in low concentrations for only a short period. In contrast to the extensive GI metabolism described for netobimin, only a low proportion of febantel is bioconverted by sheep and cattle ruminal fluids (Beretta et al., 1987). Hepatic metabolism appears to be the main site of febantel conversion into fenbendazole, after its oral administration to different animal species. The active molecule of the pro-BZD thiophanate is an ethyl BZD carbamate metabolite known as lobendazole (Delatour et al., 1988), formed in the liver with conversion rates of 34% (sheep), 52% (goats), and 57% (cattle). Thiophanate is currently used as an antifungal agent (thiophanate-methyl) for plants.

Hepatic and extrahepatic oxidative metabolism: The metabolism of BZD heavily depends on the substituent present at position 5- of the BZD ring system and involves a variety of reactions. Phase I reactions have been observed at position 5-. Hydroxylations of thiabendazole, albendazole, and fenbendazole have been demonstrated in different animal species. The sulfoxidation of albendazole and fenbendazole at the sulfur atom of the substituent group at the carbon 5- of the BZD nucleus have been widely investigated. The oxidations of albendazole to albendazole sulfoxide and fenbendazole to oxfendazole have been shown to be catalyzed by the liver microsomal mixed function oxidases in different species (Galtier et al., 1986a; Souhaili El Amri et al., 1987; Montesissa et al., 1989; Lanusse et al., 1993b; Virkel et al., 2004). Additionally, the anthelmintically active sulfoxide derivatives undergo a second, slower and irreversible oxidative step, forming the inactive sulfone (albendazole sulfone and fenbendazole sulfone) metabolites, which are also found in the bloodstream and tissues after administration of their respective parent sulfides.

The substitution of the BZD ring in position 5- has been particularly important in determining the metabolic fate of BZD methylcarbamates. This position is metabolically labile and has permitted retardation of the biotransformation of 5-substituted BZD anthelmintics as well as improvement of their efficacy (Hennessy et al., 1989). The nature of this substitution at position 5- markedly influences the sequence of BZD liver metabolism. Aromatic BZD derivatives, such as fenbendazole and oxfendazole, require more extensive hepatic oxidative metabolism than aliphatic derivatives (albendazole and albendazole sulfoxide) to achieve sufficient polarity for excretion (Hennessy, 1993). Consequently, the rates of albendazole liver microsomal sulfoxidation in sheep and cattle were higher than those observed for fenbendazole (Virkel et al., 2004). For this reason, low fenbendazole concentrations are recovered in plasma following its oral/intraruminal administration to sheep and cattle, whilst albendazole is not detected in the bloodstream after its administration as the parent drug in both species. Moreover, longer plasma mean residence times and elimination half-lives for fenbendazole and its metabolites, compared to those of albendazole metabolites, were observed in sheep and cattle (Lanusse et al., 1995) (Figure 39.3).

The involvement of the cytochrome P450 (CYP) and flavin monooxygenase (FMO) systems on the liver sulfoxidation of albendazole has been demonstrated in different animal species, including sheep (Galtier et al., 1986a) and cattle (Lanusse et al., 1993b). It has been demonstrated that FMO-mediated sulfoxidation accounted for up to 60% of albendazole sulfoxide production from albendazole, whilst CYP contributed 40% in both sheep

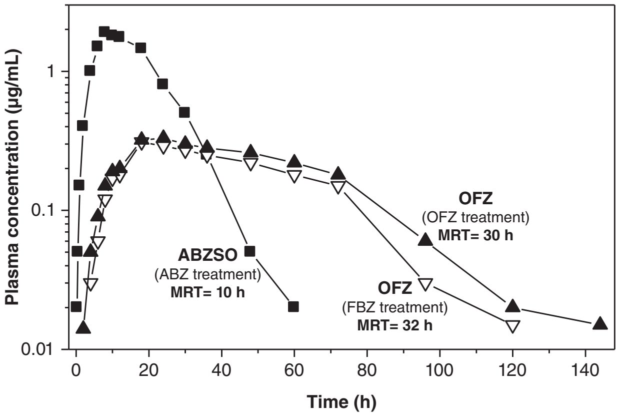


Figure 39.3 Comparative mean (n = 5) plasma concentrations of the benzimidazole sulfoxide metabolites, albendazole sulfoxide (ABZSO) and oxfendazole (OFZ), obtained after oral administration of albendazole (ABZ), fenbendazole (FBZ), and OFZ (5 mg/kg) to adult sheep (see the text for further explanation). MRT, mean plasma residence time (h) for each molecule. Source: Adapted from Lanusse et al., 1995. Reproduced with permission of John Wiley & Sons.

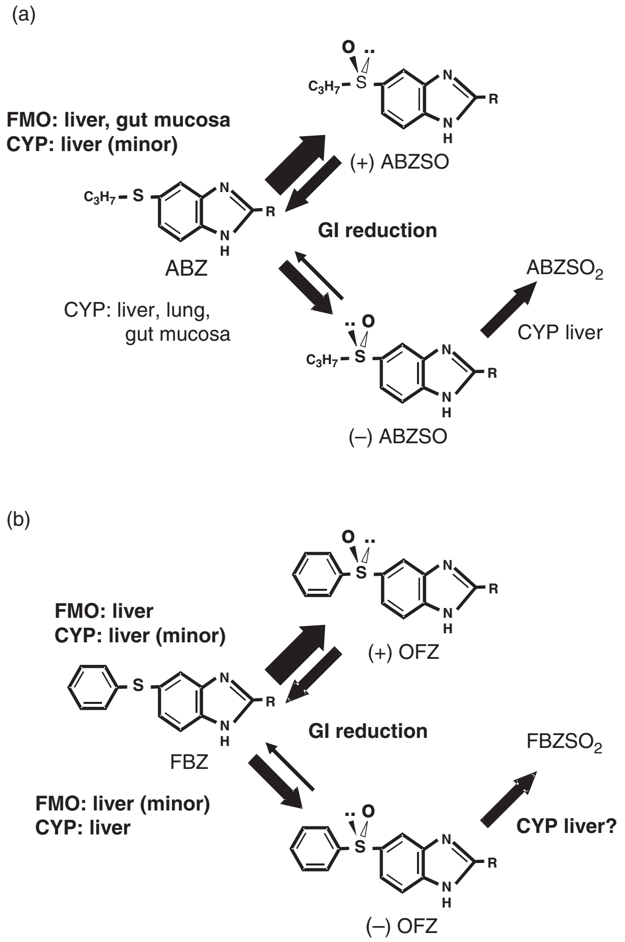


Figure 39.4 Proposed metabolic pathways for the enantioselective sulfoxidation and sulfonation of (a) albendazole (ABZ) and (b) fenbendazole (FBZ). The gastrointestinal (GI) sulforeduction of both sulfoxide enantiomers (ruminants) to form the parent thioethers (ABZ, FBZ) is also shown. The width of the arrows represents the magnitude of each metabolic pathway (see the text). FMO, flavin monooxygenase; CYP, cytochrome P-450; ABZSO, ABZ sulfoxide; OFZ, oxfendazole, FBZ sulfoxide; ABZSO$_2$, ABZ sulfone FBZSO$_2$, FBZ sulfone. Source: Data from Delatour et al., 1991; Benoit et al., 1992; Redondo et al., 1999; Virkel et al., 2002, 2004.

and cattle liver microsomes (Virkel et al., 2004) (Figure 39.4). Similarly, FMO was estimated to be the main enzymatic system involved in the liver sulfoxidation of fenbendazole (~80%) in both species (Virkel et al., 2004). Inhibition of CYP-mediated sulfoxidation by piperonyl butoxide demonstrated the participation of this enzymatic system in the hepatic metabolism of fenbendazole in horses (McKellar et al., 2002).

Both sulfoxide metabolites (albendazole sulfoxide and oxfendazole) have an asymmetric center in the sulfur atom of their side chains. This nucleophilic sulfur atom is attached to four different functional groups, which results in an asymmetric molecule nonsuperimposable with its mirror image. Thus, two different enantiomers of albendazole sulfoxide and oxfendazole have been identified (by chiral separation) in the plasma of sheep and cattle treated with albendazole and fenbendazole (prochiral molecules), respectively (Delatour et al., 1990a; Sánchez et al., 2002). It has been shown that (+)albendazole sulfoxide is the main enantiomeric form recovered in plasma and tissues at parasites locations (Alvarez et al., 2000) following albendazole treatment of sheep (Figure 39.5) and cattle. The same pattern was observed after albendazole sulfoxide administration to cattle (Cristofol et al., 2001). Most importantly, the (+)albendazole sulfoxide enantiomer seems to have a higher anthelmintic activity than that observed for the (−) enantiomer (Paredes et al., 2013). Similarly, (+)oxfendazole prevails in the systemic circulation after both fenbendazole and oxfendazole administrations to sheep (Delatour et al., 1990a; Sánchez et al., 2002) and cattle. The observed differences in the plasma/tissues availabilities of the (+) and (−) enantiomeric forms were attributed to the relative contribution of the FMO and CYP-dependent oxygenases to albendazole and fenbendazole hepatic sulfoxidation. FMO and CYP are known to be oppositely enantioselective (Cashman, 1998). FMO activity accounted for 94% (cattle) and 81% (sheep) of (+)albendazole sulfoxide production in liver microsomes (Virkel et al., 2004). In both species, the enantioselectivity of the hepatic FMO system towards (+)albendazole sulfoxide production was close to 100%. Moreover, it has been shown that (−)albendazole sulfoxide, rather than its (+) antipode, is the main substrate for the CYP-mediated formation of the inactive sulfone metabolite (Delatour et al., 1991; Benoit et al., 1992). On the other hand, FMO-mediated sulfoxidation of fenbendazole generated

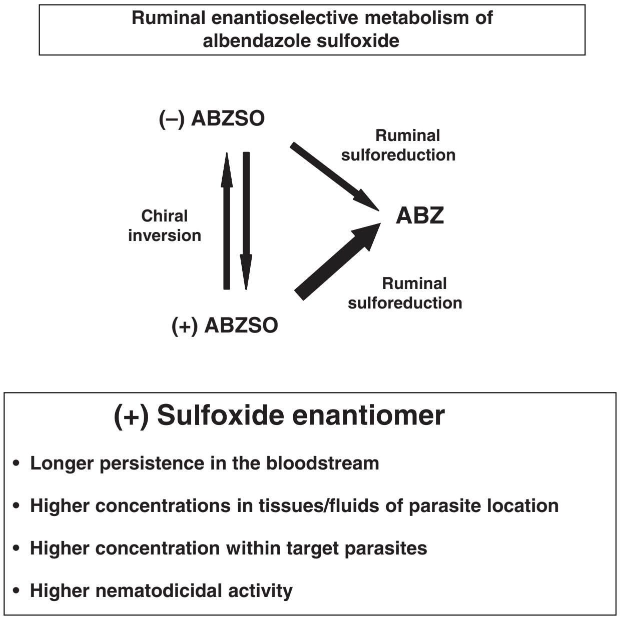

Ruminal enantioselective metabolism of albendazole sulfoxide
(–) ABZSO
Ruminal sulforeduction
Chiral inversion
ABZ
Ruminal sulforeduction
(+) ABZSO
(+) Sulfoxide enantiomer
• Longer persistence in the bloodstream
• Higher concentrations in tissues/fluids of parasite location
• Higher concentration within target parasites
• Higher nematodicidal activity

Figure 39.5 Schematic representation of the ruminal biotransformation of albendazole sulfoxide (ABZSO) enantiomers. The width of the arrows indicates the magnitude of the metabolic reactions. The main pharmacological features of the (+) sulfoxide enantiomer (compared to the (–) isoform) are listed in the bottom box. The enantioselective ruminal sulforeduction of the (+) isoform is the most relevant metabolic reaction for the formation of the parent thioether (ABZ). The same pattern is applicable to the sulforeduction of oxfendazole (OFZ) enantiomers into fenbendazole (FBZ) (see the text for detailed explanation). Source: Adapted from Virkel et al., 2004. Reproduced with permission of the American Society for Pharmacology and Experimental Therapeutics.

both (+) and (–)oxfendazole enantiomers. Fenbendazole oxidation was enantioselective towards the production of (+)oxfendazole in both sheep (65%) and cattle (79%). In conclusion, the sulfoxide metabolite recovered in plasma and target tissues after either albendazole or fenbendazole administration exists as two different chemical entities: the (+) and (–) enantiomeric forms (Figure 39.4).

Biotransformation takes place predominantly in the liver, although metabolic activity is apparent in extrahepatic tissues such as lung parenchyma and small intestine mucosa. Large quantities of albendazole parent drug were recovered from tissues at parasite locations in both sheep and cattle. Similarly, fenbendazole has been recovered from tissues at parasite locations following oral administration of oxfendazole to cattle. Altogether, these findings support the need to study the biotransformation of BZD anthelmintics in extrahepatic tissues such as lung parenchyma and small intestinal mucosa. The sulfoxidation of albendazole and fenbendazole by small intestine (cattle) and lung (sheep and cattle) microsomes was shown to be enantioselective (Virkel et al., 2004). Although the liver is the main site of albendazole and fenbendazole biotransformation, sulfoxidation in the intestinal mucosa and lung tissue may contribute to the presystemic metabolism of both anthelmintic drugs and should not be underestimated.

The administration of thiabendazole results in a rapid conversion of a parent compound into a 5-hidroxy thiabendazole metabolite, formed by aromatic ring hydroxylation. Although this metabolite is sufficiently polar for a rapid urinary excretion, both its unconjugated and sulfate or glucuronide conjugated forms have been found in urine of TBZ treated animals (Tocco et al., 1965; Gottschall et al., 1990).

After the administration of mebendazole to sheep, the parent drug is rapidly absorbed and metabolized into two main metabolites, which are identified in sheep plasma after mebendazole treatment (Behm et al., 1983). The main mebendazole metabolite results from carbonyl reduction to the secondary alcohol, which was also identified as its glucuronide or sulfate conjugates. Although, the specific enzymatic system responsible for mebendazole reduction is unknown, several cytosolic ketone reductases are involved in the formation of hydrosoluble metabolites from carbonyl-containing molecules. On the other hand, the combination of carbonyl reduction and carbamate hydrolysis produces the hydrolyzed metabolite of mebendazole. Hydrolysis of the carbamate group eliminates both the anthelmintic activity of the compound and its toxicity. Mebendazole rather than its metabolites appears to be the active anthelmintic molecule. Mebendazole metabolites are mainly excreted in bile.

Unlike aromatic substituted methylcarbamates, 5-aliphatic substituted such as albendazole and parbendazole, when oxidized, are sufficiently polar to be largely excreted in urine rather than undergo further conjugation and secretion in bile (Hennessy et al., 1989). For instance, following albendazole treatment to sheep only 8% of the total dose was recovered in bile as unconjugated albendazole sulfoxide and hydroxyalbendazole sulfoxide metabolites, and 6.3% as conjugated glucuronide and sulfate esters, mainly of 2hydroxy-albendazole sulfoxide and 2hydroxy-albendazole sulfone (Hennessy et al., 1989); 59% of the dose was recovered in urine of albendazole-treated cattle (Gyurik et al., 1981). On the other hand, the percentage of a fenbendazole dose in cattle recovered in urine (2.5%) was markedly lower than that recovered from feces (42%), the latter being mainly the unchanged fenbendazole parent compound (Short et al., 1987).

The parent flubendazole compound and its reduced and hydrolyzed metabolites have been identified in tissues of treated pigs, poultry, and sheep. After flubendazole intravenous administration to sheep, the parent drug was rapidly depleted from the bloodstream (Moreno et al., 2004), being detectable up to 36 hours post-treatment. The reduced metabolites (main metabolite) and hydrolyzed metabolites were rapidly detected in the bloodstream, which indicated a fast biotransformation of flubendazole to form both metabolic products. In vitro incubations with sheep liver microsomes indicated that flubendazole is converted to reduced flubendazole by the mixed-function oxidase system (Moreno et al., 2004).

Reductive Metabolism in the Gastrointestinal Tract

In comparison to the liver, where oxidative metabolism predominates, the GI microflora is very active in reductive reactions of foreign compounds, particularly those containing -nitro and -sulfoxide groups (Renwick et al., 1986; Rowland, 1986). Drug GI metabolism may be modified by the type of diet and many other factors affecting the GI bacterial reductive capacity. Orally administered compounds that are poorly absorbed from the GI tract will stand the greatest chance of undergoing metabolism by the microflora, although a large number of drug/metabolites gain entry to the gut via biliary secretion or plasma–GI distribution exchange (Figure 39.2) and will also be exposed to microbial activity. Drug metabolic processes taking place in the rumen are particularly important in ruminant therapeutics.

The metabolic sulforeduction of the BZD sulfoxide metabolites (albendazole sulfoxide and oxfendazole) to form the parent thioethers (albendazole and fenbendazole, respectively) has been shown to occur in ruminal and intestinal fluid contents from sheep and cattle (Lanusse et al., 1992b). A plasma–GI pH gradient (Lanusse et al., 1993a) and/or an active gastric secretion process (Alí and Hennessy, 1995) account for the distribution of BZD metabolites from plasma to the digestive tract lumen. Therefore, albendazole sulfoxide and oxfendazole can be reduced back to their respective thioethers by ruminal and intestinal microflora and may act as a source of albendazole and fenbendazole, respectively, in the GI tract. This GI metabolic reduction may be of primary importance for the antiparasitic efficacy of BZD thioethers. Since the thioethers have a greater affinity for parasite tubulin than the sulfoxides (Lacey, 1990; Lubega and Prichard, 1991), this bacteria-mediated reduction may have significant importance for the efficacy against GI parasites. Thus, the high efficacy of albendazole sulfoxide and oxfendazole against GI parasites may depend on this bacterial reduction of the sulfoxide to the more pharmacologically active thioethers.

The ruminal sulforeduction of both albendazole sulfoxide and oxfendazole is enantioselective, the (+) antipode being the main substrate to form the more active parent sulfide in the rumen (Virkel et al., 2002) (Figure 39.5). Chiral inversion is the metabolic process by which one enantiomer is transformed into its antipode. The chiral inversions of (+) into (−), but also from (−) into (+) enantiomeric forms, were observed when both enantiomers were incubated separately (as pure substrates) with ruminal fluid obtained from both sheep and cattle (Virkel et al., 2002) (Figure 39.5). Thus, the reported data suggest that chiral inversion of albendazole sulfoxide and oxfendazole enantiomers is likely to be bidirectional and may depend on either the metabolic transformation of one enantiomer into its antipode or through the formation of parent thioether (albendazole or fenbendazole) as an intermediate metabolic product. Overall, the work published by Virkel et al. (2002) demonstrates that the ruminal sulforeduction of both albendazole sulfoxide and oxfendazole is enantioselective, the (+) antipode being the main substrate to form the more active parent sulfide in the rumen.

Distribution to Parasite Location Tissues

Release from the dosage form and absorption precede entry of any anthelmintic molecule into the bloodstream, which serves as the tissue in which drug and metabolite molecules are conducted to various parts of the body. Within the bloodstream, a fraction of most drugs binds reversibly to plasma proteins, and the remainder undergoes simultaneous distribution, metabolism, and excretion. The access of BZD molecules to intracellular sites depends upon their ability to penetrate the capillary endothelium and to cross the cell membrane. Most anthelmintics are weak organic acids or bases and exist in solution, at physiological pH, as both nonionized and ionized forms; while the poor lipophilicity of the ionized molecules excludes them from passive diffusion, the lipid-soluble nonionized moieties passively diffuse across biological membranes until equilibrium is established. Once the BZD molecule has been absorbed from the GI tract, it is rapidly distributed by the circulatory system throughout the entire body. During this time, the metabolic processes necessary to facilitate its elimination commence. Extensive tissue distribution of albendazole and its metabolites in sheep (Alvarez et al., 1999) and cattle (Sánchez et al., 1997) have been reported. However, differential distribution patterns among BZD sulfides (albendazole, fenbendazole), sulfoxide (albendazole sulfoxide, oxfendazole), and sulfone (albendazole sulfone, fenbendazole sulfone) metabolites, based on their differential lipophilicities, may be expected. The distribution rate, which is indicated by the apparent volume of distribution, depends on molecular weight, lipid solubility, and plasma protein binding of each drug/metabolite. The majority of BZD compounds show a binding of less than 50% to plasma protein, relatively high volume of distribution, and a relatively fast elimination rate in ruminant species.

The rate of absorption, metabolism, and excretion of BZD anthelmintics varies from drug to drug, with slower absorption and prolonged recycling between enteral and parenteral tissues being relevant to enhance efficacy. Worms attached to the lining of the gut may be more exposed to this recycling drug than to that actually passing down the GI tract in food that is being digested. Fenbendazole, oxfendazole, and albendazole are less water soluble than earlier members of the group and, therefore, their dissolution rate, passage along the GI tract, and absorption into the systemic circulation are markedly slower compared to thiabendazole. These more lipophilic substituted BZD methylcarbamate compounds remain in the bloodstream for a longer time and, since it is assumed that an equilibrium exists between plasma and GI tract, the period of exposure of GI nematodes to effective drug/metabolites concentrations is extended. In NTB-treated cattle, peak concentrations of albendazole sulfoxide and albendazole sulfone are reached at 7–10 hours (albendazole sulfoxide) and at 15–22 hours (albendazole sulfone) posttreatment, followed by a well-defined decline in concentration in both plasma and GI compartments. However, whereas plasma concentration fell to undetectable levels (30–36 hours posttreatment), the profile of these metabolites in the rumen, abomasum, and ileum showed an "extra" slow elimination phase that extended to 72 hours posttreatment (Figure 39.6). This pharmacokinetic behavior is particularly clear for the albendazole sulfoxide and sulfone metabolites, whose elimination half-lives in both the abomasum and ileum were significantly longer compared to plasma. This metabolite-distribution process, also described for fenbendazole metabolites in cattle, may be driven by a plasma/GI tract pH gradient. The ratio of nonionized to ionized forms depends upon the pKa of the drug and the pH of the fluid in which the drug is dissolved, and the pH gradient between plasma and different tissues dictates the concentrations of drug/metabolite at either side of the separating cell membranes; at equilibrium, there will be a higher total concentration of the drug on the side of the membrane where the degree of ionization is greater. For instance, albendazole sulfoxide is an amphoteric compound which can act as either acid or base. Its pKa values are 3.45 and 9.8 for the acidic and basic groups, respectively. At plasma pH, there will be an important proportion of this molecule under the nonionic form, which will facilitate its passive diffusion from plasma to parasite location tissues. A greater plasma/abomasum pH gradient compared with that of the rumen and ileum would produce a strong ionic trapping effect (Figure 39.6), which would account for the significantly higher concentrations of albendazole metabolites found in the abomasum in comparison with plasma and other GI compartments. In addition, although albendazole (the most potent anthelmintic molecule) is not detected in the bloodstream, it has been recovered in different GI compartments, with particularly high concentration profiles recovered in the abomasal mucosa as well as from *Haemonchus contortus* recovered from treated sheep (Figure 39.7). The extensive distribution of BZD methylcarbamates from the bloodstream to the GI tract and other tissues may contribute to anthelmintic efficacy against parasites localized in body tissues including GI mucosa, GI lumen, and lungs (Alvarez et al., 1999, 2000). The recovery of both albendazole and fenbendazole parent compounds from GI mucosa and lung tissue after intravenous administration of their sulfoxide derivatives to cattle (as described in Section Reductive Metabolism in the Gastrointestinal Tract), is further evidence of the complex relationship among drug distribution, metabolism, and efficacy of BZD anthelmintics in ruminants.

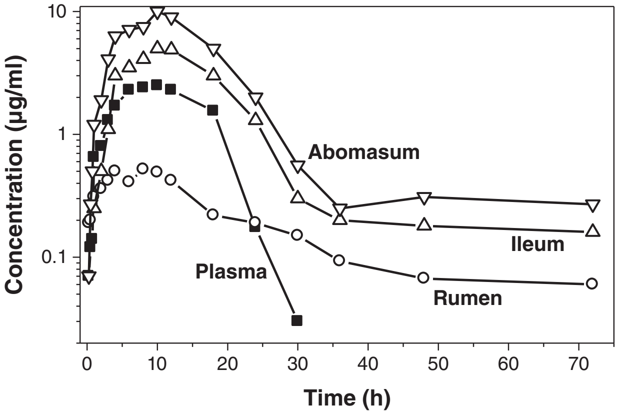


Figure 39.6 Comparative albendazole sulfoxide (ABZSO) concentration profiles measured in the bloodstream and in different gastrointestinal compartments after the oral administration of netobimin prodrug (20 mg/kg) to cattle. The results shown the slow elimination phase observed in digestive compartments (compared to plasma), which is critical for efficacy against gastrointestinal nematodes (see the text for further explanation). ABZSO elimination half-life values were: 2.29 hours (plasma), 16 hours (rumen), 34 hours (abomasum), and 67 hours (ileum). Source: Adapted from Lanusse et al., 1993a. Reproduced with permission of John Wiley & Sons.

Comparative studies have revealed considerable differences between ruminant species regarding the pharmacokinetics of anthelmintic drugs. Longer residence times for albendazole sulfoxide in sheep than in cattle, and higher plasma AUC ratios for albendazole sulfone/albendazole sulfoxide in cattle, have been consistently reported. Since sheep liver microsomes has a higher albendazole sulfoxidase activity compared with that of cattle, the greater capacity of sheep GI fluids to reduce albendazole sulfoxide back to albendazole may explain the observed species-related pharmacokinetic differences. On the other hand, the oxfendazole AUC after oxfendazole administration to goats was 39% smaller compared to that observed in sheep (Hennessy et al., 1993a). It is suggested that goats possess a faster hepatic metabolism than sheep, resulting in more rapid elimination of oxfendazole. This lower availability may account for the poor efficacy reported for oxfendazole

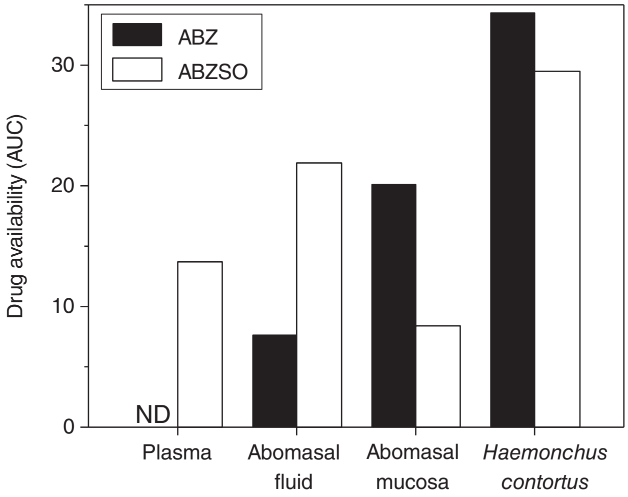


Figure 39.7 Comparative albendazole (ABZ) and albendazole sulfoxide (ABZSO) availabilities (expressed as area under the concentration vs. time curve, AUC), obtained in plasma, abomasal fluid, abomasal mucosa, and *Hemonchus contortus* recovered from infected sheep treated with ABZ (intraruminal at 7.5 mg/kg) (see the text for further explanation). ND, not detected. AUC values are expressed in μg.h/g. Source: Data from Alvarez et al., 2000.

- *ABZSO was the main active molecule measured in plasma after ABZ treatment in sheep*
- *High concentrations of ABZ and ABZSO were recovered from H. contortus*
- *ABZ total availability was greater in H. contortus compared to abomasal tissue/fluid*

given to goats at the dose recommended for sheep. These observations suggest that extrapolated data can be misleading in predicting adequate dosages and withdrawal times.

Pharmacokinetics in nonruminant species

The oral administration of BZD (fenbendazole, oxfendazole) compounds in horses demonstrated that reduced bioavailability and shorter residence times are obtained in comparison to those observed in ruminants. Following oral administration of either fenbendazole or oxfendazole (10 mg/kg) to horses, the inactive fenbendazole sulfone metabolite is the predominant analyte measured in plasma, with AUC ratios oxfendazole/fenbendazole/fenbendazole sulfone of approximately 3/1/9 (oxfendazole treatment) and 1/4/7 (fenbendazole treatment) (McKellar et al., 2002). In both cases, the time to reach the peak plasma concentration of the active oxfendazole metabolite was approximately 9 hours, which is earlier compared to that observed in ruminants (21–29 hours) and agrees with the reduced systemic availability observed in horses. Following oxibendazole oral administration to horses (10 mg/kg), parent oxibendazole could only be detected in plasma up to 1 hour posttreatment; an unidentified metabolite was recovered in the bloodstream between 0.5 and 72 hours (Gokbulut et al., 2002). Oxibendazole in vitro conversion into its metabolite was significantly inhibited by coincubation with the cytochrome P450 inhibitor piperonyl butoxide. These findings indicate that a first-pass metabolism may reduce oxibendazole systemic availability in horses.

As described for ruminant species, albendazole is not detected in plasma after its oral administration to pigs (Alvarez et al., 1995). Albendazole sulfoxide and albendazole sulfone are the main metabolites recovered from plasma up to 48 hours posttreatment. The pattern of plasma metabolites is similar to that reported after the administration to ruminant species and there appear to be no major differences between the metabolic patterns of albendazole observed in pigs and sheep. However, marked differences in the metabolism of albendazole between cattle and pigs have been described, with predominance of the sulfoxide metabolite over albendazole sulfone in pig plasma, while the inverse is observed in cattle after albendazole treatment. Similar to the observations in ruminants, the time of residence of the albendazole suspension at the acidic pH of the stomach in the pig affects its dissolution rate and the subsequent absorption of the drug from the gut. Any factor that influences gastric emptying rate may have a profound effect on the rate and extent of BZD absorption in monogastric animals. After oral fenbendazole administration to pigs, oxfendazole (the main metabolite recovered) and fenbendazole sulfone are rapidly detected in plasma up to 48 hours posttreatment. Oxfendazole reaches a C_{max} value (0.7 μg/ml) at 12.5 hours posttreatment (Petersen and Friis, 2000). The bioavailability of fenbendazole in pigs after its oral administration was estimated to be about 30%. This low value is a consequence of the low water solubility of fenbendazole and its poor GI absorption, plus the significant liver first-pass effect on the drug. Interestingly, fenbendazole was measured in the bloodstream of oxfendazole-treated pigs. Its presence could be explained by oxfendazole reduction, which probably takes place in the pig large intestine, where an important microbial activity is present, as it is the main site of cellulose degradation in this species.

The gut transit time influences the dissolution and absorption of poorly water-soluble drugs such as the BZD anthelmintics. The drugs that do not dissolve in GI contents pass down and are excreted in the feces without exerting their action. The GI physiology and the digesta transit time are markedly different between monogastric species and ruminants. This is relevant for dogs and cats, whose GI transit times are significantly shorter compared to ruminant species; this has a marked effect on the absorption, kinetic behavior, and efficacy of BZD anthelmintics in companion animals. Albendazole, albendazole sulfoxide, fenbendazole, febantel, or mebendazole, formulated as tablets or suspensions for oral administration in dogs and cats, must dissolve at low pH (stomach), and it has been demonstrated that the dissolution rate may be altered according the size of the formulation particle (Hennessy, 1993). Several studies suggest that only limited rates of dissolution and absorption of BZD anthelmintics are achieved in the cat, dog, and man. Consequently, these compounds may need to be given at a higher dose or as multiple administrations in order to maintain therapeutic concentrations and, therefore, to achieve acceptable anthelmintic efficacy (Roberson and Burke, 1982; Edwards and Breckenridge, 1988).

Mebendazole is poorly absorbed after oral administration and only low concentrations (representing 10% or less of the total administered dose) of mebendazole parent drug and its inactive metabolite, hydroxymebendazole, were recovered in plasma of humans and dogs after oral treatment (Witassek et al., 1981; Edwards and Breckenridge, 1988). This observation could explain the lack of efficacy of mebendazole against lung parasites in humans and dogs and the need for multiple dose treatments. The pharmacokinetics of fenbendazole parent drug and its metabolites in dogs has been characterized (McKellar et al., 1990). Fenbendazole parent drug, its active sulfoxide (oxfendazole), and inactive sulfone (fenbendazole sulfone) metabolites were recovered in plasma for 48 hours after administration of a single oral dose of 20 mg/kg. Interestingly, plasma concentrations for both active molecules (fenbendazole and oxfendazole) are depleted in parallel from the body, giving an AUC ratio (fenbendazole : oxfendazole) of approximately 0.82. This may result in exposure of target parasites to the fenbendazole moiety (which has greater potency than oxfendazole), accounting for some advantageous activity against the tissue-arrested larvae and other immature parasite stages (McKellar et al., 1990). When albendazole was given as a single dose in tablet form, albendazole sulfoxide and albendazole sulfone were the main metabolites detected in plasma for up to 16 hours posttreatment (Sánchez et al., 2000a). Compared to ruminants, where the rumen acts as drug reservoir, effective treatment in monogastrics requires more prolonged, multidose regimes of at least 3 to 5 days, depending on the dose used. There are conclusive pharmacokinetic results to support the concept that higher efficacy could be expected in dogs and cats by increasing the number of treatments rather than increasing the dose. In fact, the AUC of fenbendazole in dogs was similar after a single administration of fenbendazole at different dose levels over a range between 2.5 and 100 mg/kg (McKellar et al., 1993). Lower fenbendazole plasma concentrations were achieved in dogs when fenbendazole was given on an empty stomach, compared with its administration given in food. However, fat content in the diet does not affect the absorption of fenbendazole after its oral administration to dogs (McKellar et al., 1993).

After the oral administration of fenbendazole to chickens, the parent drug, oxfendazole and fenbendazole sulfone, were detected in plasma, fenbendazole sulfone being the main metabolite recovered up to 96 hours posttreatment (Taylor et al., 1993).

A new formulation albendazole sulfoxide–base (ricobendazole) has been marketed for use in dogs in Latin America. Interestingly, this formulation showed around 500% increase in AUC and C_{max} values when compared to the conventional albendazole tablet formulation (Dib et al., 2011). This increased systemic exposure correlated with enhanced efficacy against most GI helminth parasites, from which this formulation is recommend as a single dose treatments. The improved efficacy obtained at a single (20 mg/kg) dose may be based on the high solubility of albendazole sulfoxide at the different digestive tract pHs compared to albendazole parent drug.

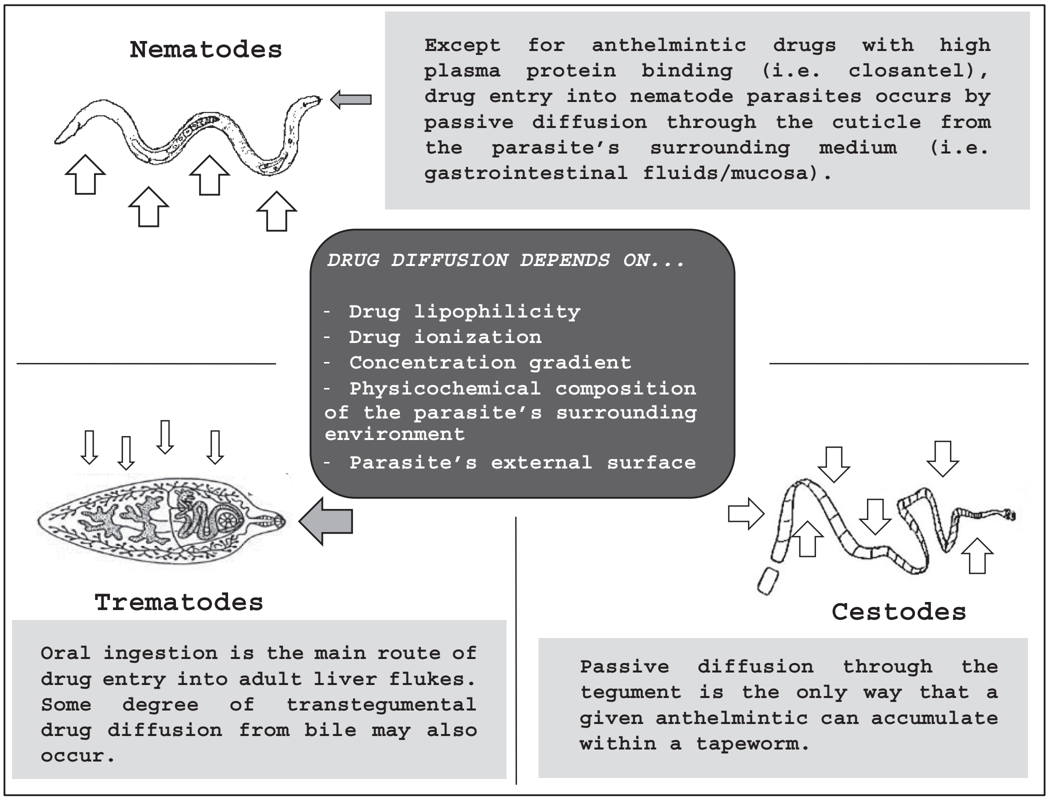
Albendazole parent drug is absorbed slowly and detected between 1 and 6 hours posttreatment in chickens, albendazole sulfoxide being the main metabolite detected, with a peak concentration of 0.83 μg/ml obtained at 1 hour posttreatment (Csiko et al., 1996) and low albendazole sulfone concentrations measured between 2.5 and 30 hours after albendazole administration. Interestingly, while in other animal species, such as pigs, dogs, and ruminants, albendazole is not detected in plasma after its oral administration, the parent drug was measured in hens. This finding has been associated with a fast absorption process and/or a slow metabolic rate in hens compared with other species (Bistoletti et al., 2013). Additionally, while drug absorbed at either proventriculus, gizzard, or intestines reaches the liver drained by the venous portal system, albendazole absorbed at the crop reaches the systemic circulation through the jugular vein, avoiding the liver "first-pass" effect. Data provided for chickens indicated that albendazole and fenbendazole are metabolized in avian species via the same metabolic pathway as in mammals (McKellar and Scott, 1990). As can be expected for dogs, cats, and man, the GI reductive metabolism of the BZD sulfoxide into the parent sulfides is markedly less relevant in poultry compared to ruminant species. Flubendazole is widely used as an anthelmintic in poultry and its main metabolites, hydrolyzed flubendazole and reduced flubendazole, have been identified in tissues of flubendazole-medicated turkeys.

Mechanisms of Drug Transfer into Target Parasites

Understanding the role of the cuticular/tegumental structure on the process of drug uptake by helminth parasites is an area of major scientific interest. It has been shown from in vivo and ex vivo studies that transcuticular diffusion is the predominant pathway for the entry of anthelmintic drugs into nematodes (Cross et al., 1998; Alvarez et al., 2001). Although, there are relevant structural differences between the external surface of nematodes (cuticle) and cestodes/trematodes (tegument), the mechanism of drug entry to both types of helminths seems to be equally dependent on lipophilicity, as a major physicochemical determinant of drug capability to reach therapeutic concentrations within the target parasite. A high correlation between the octanol–water partition coefficients for different BZD anthelmintics and their capacity to diffuse through the tegument of *Moniezia expansa* has been shown (Mottier et al., 2003). The most lipophilic BZD compounds (fenbendazole, albendazole, mebendazole) (partition coefficients >3.7) were measured at higher concentrations within the tapeworm compared to those with the lowest lipid–water partition values (oxfendazole, albendazole sulfoxide, thiabendazole, etc.). A lower diffusion of albendazole and albendazole sulfoxide into *Ascaris suum* compared to *Moniezia* spp. and *Fasciola hepatica* has been demonstrated (Alvarez et al., 2001). Nematodes maintain a strongly buffered environment in the aqueous spaces of the cuticle structure, with a pH value of this compartment around 5.0 (accumulation of organic acids as products of carbohydrate metabolism). The lipid-containing region of the cuticle represents the rate-determining barrier for passive transport: the molecular size, restriction by pores, the intrinsic lipid–water partition coefficient, and pH/pKa relationship are among the rate-determining factors (Ho et al., 1992). BZD molecules largely exist in their ionized form at the acidic environment of the nematode surface, which may limit their diffusion across the cuticle. This ionization-mediated impairment of drug diffusion and the complex structure of the nematode cuticle compared to the cestode/trematode tegument, may explain the drug penetration differences observed between nematode and other helminths (Alvarez et al., 2001). In fact, this work demonstrated that the diffusion of BZD molecules into *Fasciola hepatica* and *Moniezia* spp. is markedly greater than that observed in the nematode *Ascaris suum* maintained under the same ex vivo conditions (Figure 39.8).

The identification of the capability of different helminth parasites to biotransform anthelmintic drugs is considered as another crucial step in the overall interpretation of the pharmacological activity of these drugs. It has been shown that both cytosolic and microsomal fractions obtained from different helminths are able to oxidized albendazole into its sulfoxide derivative. While this oxidative pathway is predominant in *Fasciola hepatica*, the sulforeduction of albendazole sulfoxide into its parent sulfide compound is the main metabolic activity identified in tapeworms (Solana et al., 2001). Additionally, the ex vivo biotransformation of albendazole to different albendazole glucoside metabolites was significantly higher in albendazole-resistant adult *Haemonchus contortus* compared to susceptible worms (Vokřál et al., 2013). The altered activities of certain detoxifying enzymes might partly protect the worms against the toxic effect of the drugs, contributing to a drug resistance mechanism. Although further investigation is required to establish the pharmacological relevance of drug biotransformation in target parasites, particularly regarding its potential impact on the development of resistance, these findings are complementary to studies addressing the understand of mechanisms of drug accumulation in helminths.

Higher albendazole sulfoxide and oxfendazole compared to albendazole and fenbendazole concentrations have been reported in GI fluids of different ruminant species. However, the high lipophilicity of the parent thioethers (albendazole, fenbendazole) assures their penetration through the external surface of parasites. In fact, higher concentrations of albendazole were measured in *Haemonchus contortus* recovered from infected treated sheep compared to those of its sulfoxide metabolite (albendazole sulfoxide) (Alvarez et al., 2000). Since albendazole was not recovered in peripheral plasma, only drug from the pool found in abomasal fluid and mucosal tissue was available to reach the target nematode through its external cuticle. It is also likely that *Haemonchus contortus* may feed on portal blood. However, the low albendazole concentrations recovered in portal blood would not explain the large amount of albendazole recovered from this abomasal parasite. These findings may confirm the relevance of the transcuticular diffusion process, even in a blood-sucking parasite such as *Haemonchus contortus*. The higher anthelmintic potency of albendazole and fenbendazole and their greater capability to diffuse into the parasite, suggest that the parent drug may be the main molecule responsible for the activity against GI parasites. However, a complementary effect of the sulfoxide metabolites may contribute to the overall anthelmintic activity of these BZD compounds. On the other hand,



Except for anthelmintic drugs with high plasma protein binding (i.e. closantel), drug entry into nematode parasites occurs by passive diffusion through the cuticle from the parasite's surrounding medium (i.e. gastrointestinal fluids/mucosa).

DRUG DIFFUSION DEPENDS ON...

- Drug lipophilicity
- Drug ionization
- Concentration gradient
- Physicochemical composition of the parasite's surrounding environment
- Parasite's external surface

Oral ingestion is the main route of drug entry into adult liver flukes. Some degree of transtegumental drug diffusion from bile may also occur.

Passive diffusion through the tegument is the only way that a given anthelmintic can accumulate within a tapeworm.

Figure 39.8 Main routes of in vivo drug entry into different target helminth parasites.

transcuticular transfer appeared to be the main route of passage of other anthelmintic compounds, such as ivermectin, into the filarial nematode *Onchocerca ochengi* (Cross et al., 1998) and *Haemonchus contortus* (Lloberas et al., 2012).

Drug concentration at the site of parasite location and the lipid solubility of the anthelmintic molecule are not the only parameters to consider when drug kinetics are evaluated. The physicochemical characteristics of the tissues and fluids surrounding the parasite may play a relevant role in drug accumulation. The partitioning of the active drug/metabolites between an aqueous gastrointestinal fluid and the lipoidal tissue of the parasite may facilitate the accumulation of the drug within the parasite. This drug partitioning phenomenon may be different for other sites of parasite location such as the biliary tract, where the bile-induced micelle formation may affect the diffusion of the active drug/metabolite into the target parasite (i.e., *Fasciola hepatica*). The physicochemical features of the environment where the target parasite is immersed play a pivotal role in the process of drug access, indicating that some helminths may be protected from the deleterious effect of an anthelmintic drug when living in their predilective location. This phenomenon may also explain many therapeutic failures observed in parasite control in both human and veterinary medicine, which in some cases have contributed to exposure of target parasites to subtherapeutic drug concentrations.

Anthelmintic Spectrum

BZD and pro-BZD were introduced into the animal health market primarily for the control of GI nematodes, not only for use in livestock animals (cattle, sheep, goats, swine, and poultry), but also for horses, dogs, and cats. The use of BZD and pro-BZD compounds quickly became widespread because they offered major advantages over previous available drugs in terms of spectrum, efficacy against immature stages and safety for the host animal. Additionally, BZD anthelmintics have ovicidal activity. With the exception of the halogenated BZD thiol, triclabendazole, which has only flukicidal activity against all stages of *Fasciola hepatica* (see Chapter 40), all BZD and pro-BZD can be classified as broad-spectrum anthelmintics. However, the substituted sulfur-containing methylcarbamates (albendazole, fenbendazole, etc.) and pro-BZDs (febantel, netobimin) have an extended spectrum of activity compared to the earlier BZD thiazolyls (i.e., thiabendazole). It has been demonstrated that the greater potency and spectrum of activity of the newer BZD is largely a function of their pharmacokinetic behavior rather than entirely due to their intrinsic differences in activity. Overall, the BZD methylcarbamate are broad-spectrum anthelmintics active against a variety of GI and lung nematodes, tapeworms, and trematodes (albendazole derivatives). As a consequence of their pharmacokinetic behavior in monogastric species (particularly in dogs and cats), the BZDs are more effective when they are administered for several days, compared single-dose treatments. The available commercial formulations and indications for use (route of administration, dosage, and target animal species), may vary among different countries. Readers interested on any specific issue on the spectrum of activity for BZD compounds are referred to previous editions of this textbook (see Reinemeyer and Courtney, 2001). Some general considerations on the spectrum of activity for the most widely used BZD and pro-BZD compounds are summarized below.

Thiabendazole

Thiabendazole is indicated for the treatment and control of GI roundworms in horses, cattle, sheep, and goats, and the control of lungworms in sheep. Thiabendazole is administered orally at the dose range 66 to 110 mg/kg. It is also used as a fungicide for crop protection. In horses, thiabendazole is used as a single oral dose administered as a drench or by stomach tube, or as an oral paste. It is usually associated with piperazine to increase the efficacy against ascarids and immature *Oxyurus* spp. Thiabendazole is ineffective against cestodes or trematodes. In cattle, at the recommended dose rates, it is effective against adult and developing stages of *Ostertagi ostertagi* but not against stage EL_4. Thus, thiabendazole is not useful to prevent type II ostertagiasis. In pigs, thiabendazole has little efficacy against *Ascaris suum* and *Trichuris suis*.

Netobimin

The pro-BZD netobimin is formulated as a suspension for oral administration in cattle and sheep. It is indicated (7.5 mg/kg) for the removal and control of tapeworms (heads and segments)(*Moniezia expansa, M. benedeni*), adults and larval stages of abomasal worms (*Haemonchus contortus, H. placei, Trichostrongylus axei, Ostertagia ostertagi, Teladorsagia circumcincta, Marshallagia marshalli*), intestinal worms (*Trichostrongylus colubriformis, Chabertia ovina, Nematodirus spathiger, N. filicollis, N. helvetianus, Cooperia oncophora, C. punctata, Bunostomun phlebotomum, Oesophagostomum radiatum*), and lungworms (*Dictyocaulus filaria*, *D. viviparus*). Higher doses (20 mg/kg) are required for control of adult liver flukes (>14 weeks old) (*Fasciola hepatica, Fascioloides magna*) and *Thysanosoma actinioides*, and type II ostertagiasis (cattle).

Albendazole

Albendazole is formulated as a suspension for oral administration (as a drench) in cattle and sheep at the recommended dose of 7.5 and 5 mg/kg, respectively. It is indicated for the removal and control of a broad spectrum of helminth parasites, including: tapeworms (heads and segments), abomasal and intestinal nematodes (adults and fourth-stage larvae), and lungworms (adults and larval stages). Additionally, at 10 mg/kg (cattle) and 7.5 mg/kg (sheep), albendazole is active against adult liver flukes (>14 weeks old). Ovicidal activity has also been demonstrated at 8 hours posttreatment. Albendazole appeared to be effective in suppressing cyst excretion by *Giardia*-infected calves, at the oral dose of 20 mg/kg once daily for 3 days; it is thus a potential antigiardial drug for use in farm animals (Xiao et al., 1996). Similar results were observed in *Giardia*-infected dogs after oral administration (25 mg/kg) twice a day for 2 days (Barr et al., 1993).

Ricobendazole

Ricobendazole is the sulfoxide metabolite of albendazole and it is also available as a suspension for oral administration in cattle (10 mg/kg) and sheep (7.5 mg/kg). The spectrum of activity is equivalent to that described for albendazole, including the ovicidal effect. A novel ricobendazole injectable solution (15%) for subcutaneous administration to cattle is available in some countries. It has been successfully used to control abomasal and intestinal nematodes and lungworms. A 5 mg/kg dose rate is recommended to control the larval fourth stage of *O. ostertagi*. The formulation is not recommended for control of liver flukes and tapeworms. In Europe, ricobendazole is also approved for use in pheasants by feed administration (17 mg/kg during 3 days) and is mainly indicated for the control of ascarids and capillarid infections.

Febantel

Febantel, a pro-BZD compound, is used in ruminants, horses, dogs, cats, and pigs. It is formulated as a paste, suspension, or tablets for oral administration. It is used at a dose of 10 mg/kg (ruminants), 6 mg/kg (horses), 10 mg/kg (dogs and cats) and 20 mg/kg (pigs). Dogs and cats required a 3-day period of treatment. Since FBT has not anthelmintic activity, its spectrum depends on its main active metabolites fenbendazole and oxfendazole. Consequently, its spectrum is similar to these compounds.

Fenbendazole

Fenbendazole is formulated as a suspension for oral administration in cattle, sheep, and goats at the recommended dose of 10 mg/kg (cattle) and 5 mg/kg (sheep, goats). Fenbendazole is indicated for the removal and control of tapeworms (heads and segments), abomasal and intestinal nematodes (adults and fourth stage larvae), and lungworms (adults and larval stages). Fenbendazole also have ovicidal activity. Fenbendazole is effective for the treatment of *Giardia* infections in calves at an oral dose of 10 mg/kg administered once, or 5 mg/kg given every 24 hours for a 3-day period (O'Handley et al., 1997). Fenbendazole is administered orally to horses at 5 mg/kg for control of large strongyles (*Strongylus vulgaris, S. edentatus, S. equinus*), small strongyles (cyathostomes), and pinworms (*Oxyuris equi*). Higher doses (10 mg/kg) are required for ascarids (*Parascaris equorum*) control. At 10 mg/kg for 5 consecutive days, fenbendazole is indicated for control of arteritis caused by the fourth stage larvae of *S. vulgaris*, encysted mucosal cyathostomes larvae, including early (hypobiotic) and late third stages larvae. In pigs, fenbendazole is used as a powder (3 mg/kg per day for 3 consecutive days), and is 99% effective against *Ascaris suum* larvae migrating through the liver and lungs, and most GI nematodes (mature and immature) (*Metastrongylus apri, Oesophagostomun dentatum, O. quadrispinulatum, Hyostrongylus rubidus, Trichuris suis, Stephanurus dentatus*). Fenbendazole is used at 50 mg/kg daily for 3 consecutive days in dogs for removal of hookworms (*Ancylostoma caninum*), ascarids (*Toxocara canis, Toxascaris leonina*), whipworms (*Trichuris vulpis*), and tapeworms (*Taenia*). However, is not recommended for control of the cestode *Echinoccocus granulosus* or *Echinococcus multilocularis*. A 50 mg/kg dose for 3 days achieves good therapeutic effect against *Giardia* in dogs (Barr et al., 1993). The use of fenbendazole (granules) in zoo and wildlife animals has been approved by the Food and Drug Administration (USA). It is used at 10 mg/kg during 3 days, orally administered with food, and is indicated for the control of nematodes (ascarids, hookworms, and tapeworms) of Felinae (lion, tiger, puma, cheetah, leopard) and Ursidae (black bear, polar bear).

Oxfendazole

Oxfendazole is administered orally to cattle (4.5 mg/kg), sheep (5 mg/kg), and horses (10 mg/kg). Additionally, has been used in goats, dogs, and pigs. In all cases it is recommended for the control of the same parasites as its sulfide parent compound (fenbendazole). To avoid the ruminal bypass, an oxfendazole suspension has been approved for intraruminal administration in cattle. As described for albendazole and fenbendazole, oxfendazole can be used for the prevention and control of both type I and type II ostertagiasis in cattle. However, variable efficacy against stage EL_4 has been reported. A probable explanation for the observed variability in efficacy is the metabolic activity associated to the larval stage, where the highest efficacy could be expected during induction of inhibition and emergence of inhibited larvae and the lowest efficacy during the quiescent period. In horses, oxfendazole have a similar efficacy against GI nematodes, like the parent fenbendazole. Oxfendazole has been demonstrated to have activity against *Taenia solium* cysticercus in pigs after a single oral administration at 30 mg/kg (Gonzales et al., 1996), which has been proposed as a tool to interrupt the transmission cycle of this parasite, protecting people from neurocysticercosis. Additionally, in controlled efficacy tests using naturally infected pigs, this dosage regimen has been demonstrated to be effective against adult liver flukes (Ortiz et al., 2014) and adult stages of *Ascaris suum*, *Oesophagostomum* spp., *T. suis,* and *Metastrongylus* spp. (Alvarez et al., 2013).

Oxibendazole

Oxibendazole is used in the treatment of adult and larval stages of GI nematodes of pigs, cattle, sheep, and horses. It is used orally in suspension or paste at a dosage of 10 mg/kg (sheep and cattle) or 15 mg/kg (horses). A 10 mg/kg dose in horses is sufficient for removal and control of large strongyles, small strongyles, large roundworms, and pinworms.

Mebendazole

Mebendazole is administered orally to horses (8.8 mg/kg), sheep, and goats (15 mg/kg). It is also used in game birds, pigs, deer, dogs, and poultry as different types of formulations: premixes for medicated feed, pastes, tablets, granules, and drenches, all for oral administration. It is indicated for the control of large strongyles, small strongyles, large roundworms, and mature and immature larval stages of pinworms in horses. Doses of 22 mg/kg for 3 consecutive days are recommended for use in dogs for the treatment of roundworms, hookworms, whipworms, and tapeworms.

Flubendazole

Flubendazole is available as tablets, pastes, pellets, and premixes for oral administration in pigs, chickens, turkeys, and game birds. It is used at 5 mg/kg (single oral administration) and at 30 mg/kg in feed for 10 days in pigs, and is active against lungworms, roundworms, nodular worms, and whipworms. A 10-day feed period is required for control of heavy *Trichuris suis* infections. Oral treatment at 22 mg/kg for 3 consecutive days is recommended in dogs and cats for removal of common GI parasites and tapeworms. Flubendazole is used in poultry at 60 mg/kg in feed for 7 days (equivalent to 5 mg/kg/day for broilers and 3.6 mg/kg/day for laying hens). Flubendazole has ovicidal activity and work done in sheep demonstrates that flubendazole has a volume of distribution higher than those reported for albendazole and oxfendazole (Moreno et al., 2004), which may offer a new attractive alternative for use of the drug against tissue-dwelling parasites in sheep, where the presence of high drug concentrations for an extended period of time is usually crucial to achieve optimal clinical efficacy.

Routes of Administration and Drug Formulations

As discussed in Section Pharmacokinetic Behavior, BZD anthelmintic compounds are virtually insoluble in water, which limits most of the formulations to suspension, paste, granule, tablets, blocks, powder, and pellets for oral or intraruminal administration, or for administration in feed. Drench formulations are most frequently used in ruminant species, pastes are often preferred for horses, tablets for dogs and cats, and feed administration (powder) for swine and poultry. BZD are generally administered to ruminants in the form of a single oral dose. This single-dose treatment has traditionally been done by oral drench or by intraruminal injection; this latter approach is based on the design of a special syringe (intraruminal injector) that ejects the drug directly into the rumen cavity. This device is commercially available for the administration of a concentrated suspension of oxfendazole to cattle and was mainly designed to overcome the potential problems of the esophageal groove closure after oral treatment (see Section Factors Affecting the Disposition Kinetics and Efficacy of Benzimidazole Anthelmintics). To reduce the cost associated with treating large number of animals and with growing evidence that divided anthelmintic doses and prolonged administration increase anthelmintic efficacy, several methods for drug delivery have been used in the past. The incorporation of drug into feed blocks, for ingestion of small amounts over a prolonged grazing period, and the inclusion of drugs in drinking water, have been used for therapeutic and prophylactic parasite control. Convenience and labor saving are obvious with these group-medication systems, but at the same time no direct control is possible over the drug intake rate of individual animals.

The most versatile technology in anthelmintic drug delivery has been the development of ruminal devices that, when given to individual animals, can deliver drugs for an extended period of time. These controlled-release devices or boluses more readily provide the conditions for increased anthelmintic efficacy, such as prolonged exposure of parasites to sustained concentrations of active parent drugs or their metabolites. However, due to the high selection pressure, this sustained exposure may facilitate the development of drug-resistance parasite populations. After oral administration, the boluses remain in the rumen-reticulum and release the drug over a long period of time, either in a sustained or pulsatile manner. Different controlled-release systems have been developed for the delivery of BZD anthelmintics. The oxfendazole pulse release bolus (Rowlands et al., 1988) is based on principle of releasing a series of individual therapeutic doses of oxfendazole at predetermined intervals (20–21 days), which approximately coincide with the prepatent period of the major parasites nematodes of cattle, for about 4 months. This is achieved by the continuous galvanic corrosion of a magnesium alloy rod that periodically exposes an annular oxfendazole tablet to the ruminal fluid (Campbell, 1990). An intraruminal slow-release capsule which delivers, in the rumen of sheep and cattle, a low daily dose of albendazole for approximately 3 months has been designed. The device is a hollow cylinder of nonbiodegradable plastic which contains tablets of 3.85 g (sheep) and 18.46 g (cattle) of albendazole, and two external wings. After the administration, the wings spread out while the ruminal fluid starts to dissolve the first tablet, which releases the drug slowly in the rumen; a metal spring pushes six tablets toward the open end of the capsule. Following the administration of this device, albendazole metabolites were found in plasma for 90 days (cattle) and 105 days (sheep) after capsule administration (Delatour et al., 1990b). Albendazole is also available in controlled-release capsules, which contain 2.1 and 3.85 g of albendazole/capsule for use in weaner and adult sheep, respectively, showing a sustained anthelmintic action for 90 to 100 days after administration. Additionally, an ivermectin/albendazole capsule has been developed to assure protection against GI parasites susceptible to either compound (Castells et al., 2011).

Factors Affecting the Disposition Kinetics and Efficacy of Benzimidazole Anthelmintics

Several host-related factors may affect the kinetic behavior and resultant clinical efficacy of BZD compounds. Dissolution, absorption, and biotransformation are three of the most important processes affected by a series of host-related factors. Consult Chapter 4 in this text for background to these phenomena. Manipulation of the pharmacokinetic and metabolic patterns and the comprehension of different factors modulating them seem to be excellent alternatives to improve the use of BZD in ruminants.

Effect of ruminant esophageal groove closure: The ruminoreticulum is usually the first enteric chamber encountered by an orally administered drug. However, ingested fluids may partially bypass the ruminoreticulum to enter the abomasum via the omasum following closure of the reticular groove, a reflex especially developed in the nursing ruminant but inconsistently active in the adult. Thus, variable portions of a drug solution or suspension administered orally may became divided between the ruminoreticulum and abomasum, resulting in a complex absorption process, which may then contribute to unpredictable drug efficacy. Occasionally, reduced systemic availability and efficacy of BZD methylcarbamates have been found after oral in comparison with intraruminal administration (Hennessy and Prichard, 1981). A portion of the orally administered anthelmintic may bypass the rumen and rapidly enters the abomasum by closure of the esophageal groove. As a consequence, the poor absorption, due to insufficient time for dissolution of BZD suspension's particles, results in reduced plasma bioavailability of active BZD metabolites. Such an effect may indicate that the so-called "reservoir" and "slow delivery" effects of the rumen would be lost; although lag times between treatment and plasma detection of active metabolites could as a result be shorter, the overall bioavailability of active BZD metabolites and their resultant efficacy are significantly reduced. In spite of conflicting results and difficulties in assessing its practical implications, it is clear that upon spontaneous closure of the esophageal groove a portion of an oral drench dose may bypass the rumen, which consequently affects the kinetic behavior and clinical efficacy. It is likely that this phenomenon is more relevant for: (i) compounds that are activated in the rumen such as netobimin prodrug, or (ii) those BZD anthelmintics with a low solubility in abomasal fluid such as fenbendazole or albendazole. Thus, an important proportion of the dose of orally administered fenbendazole would directly reach the abomasum, whereby fenbendazole's insolubility and the short residence time of the digesta in this organ may account for both an inefficient and/or erratic absorption and a significant proportion of the dose passing down the GI tract to be eliminated in feces.

Effect of reduced GI transit time: Modified feeding management has been recommended to restore the anthelmintic action of those BZD compounds whose potency has been compromised by resistance (Alí and Hennessy, 1995). An enhanced plasma availability of oxfendazole, induced by temporary feed restriction in sheep, accounted for increased efficacy of the drug against BZD-resistant nematode strains (Alí and Hennessy, 1995). Fasting the animals prior to intraruminal treatment resulted in pronounced modifications to the absorption and disposition kinetics of albendazole metabolites in cattle; that is the administered drug appeared to be absorbed to a greater extent than in fed animals (Sánchez et al., 2000b). Starvation decreases digesta flow rates. A delayed GI transit time that decreased the rate of passage of the anthelmintic drug down the GI tract may have accounted for the enhanced absorption observed in fasted compared to fed animals (Figure 39.9). The fasting-induced changes to the kinetic behavior and quantitative tissue distribution of BZD methylcarbamates may have particular relevance to design strategies to increase activity against susceptible parasites. The increased concentration profiles of active drug (both parent albendazole and its sulfoxide metabolite) measured in tissues where target parasites are located (i.e., GI mucosa, lung tissue, etc.) is a strong scientific argument to recommend the "fasting approach" to improve parasite control in ruminants, and this is now recommended worldwide. However, fasting-induced improvement in BZDs dissolution/absorption may not be useful when a high level of drug resistance is already established in the treated nematode population (Alvarez et al., 2010).

Effect of the type of diet: The type of feed also influences the rate of passage of digesta and that of orally administered BZD drugs in ruminants and pigs, which may in turn affect drug absorption. The binding of different BZD compounds to dietary fiber substantially modified the duration of the so called "rumen reservoir" effect (Hennessy, 1993), altering the overall bioavailability of BZD and their metabolites in the bloodstream. Various reports using widely differing type of diets, according to local feeding conditions, have demonstrated the impact of feeding on the systemic availability of albendazole, fenbendazole, and oxfendazole in different ruminant species and pigs. The delayed GI transit time and lower abomasal pH in calves fed on a concentrate-based diet, compared to those grazing on pasture, facilitated the dissolution and absorption of albendazole administered intraruminally as a drug suspension in cattle (Sánchez et al., 1999). As a consequence, significantly higher C_{max} and AUC values were obtained in calves fed on the concentrate diet compared to those grazed on pasture. Furthermore, enhanced abomasal fluid levels of albendazole and albendazole sulfoxide were observed in concentrate-fed calves. Additionally, different types of diets have been shown to affect ruminal pH and to modify the microflora-mediated metabolic sulforeduction of BZD derivatives (Virkel et al., 1997), which further impacts on the disposition kinetics of these compounds in ruminants.

Effect of nutritional status and parasite infection: Because of its central position in the biotransformation and metabolic activities of the animal, the liver is vulnerable not only to damage by various foreign chemicals, microorganism, and parasites, but also to dietary induced metabolic disorders that may induce important changes on the pharmacokinetic behavior, side-effects, and expected efficacy of the chosen anthelmintic for therapy. The results reported by Sánchez et al. (1996) showed that the poor nutritional status of feed-restricted animals induced marked modifications to the pattern of albendazole biotransformation and its resultant disposition kinetics in cattle, which was likely produced by interference of the nutritionally induced biochemical changes on albendazole liver biotransformation. During both starvation and underfeeding in cattle, the hepatic concentrations of NADPH and ATP have been shown to decrease to about 60 and 40%, respectively, of their normal values in animals fed ad libitum. It is likely that

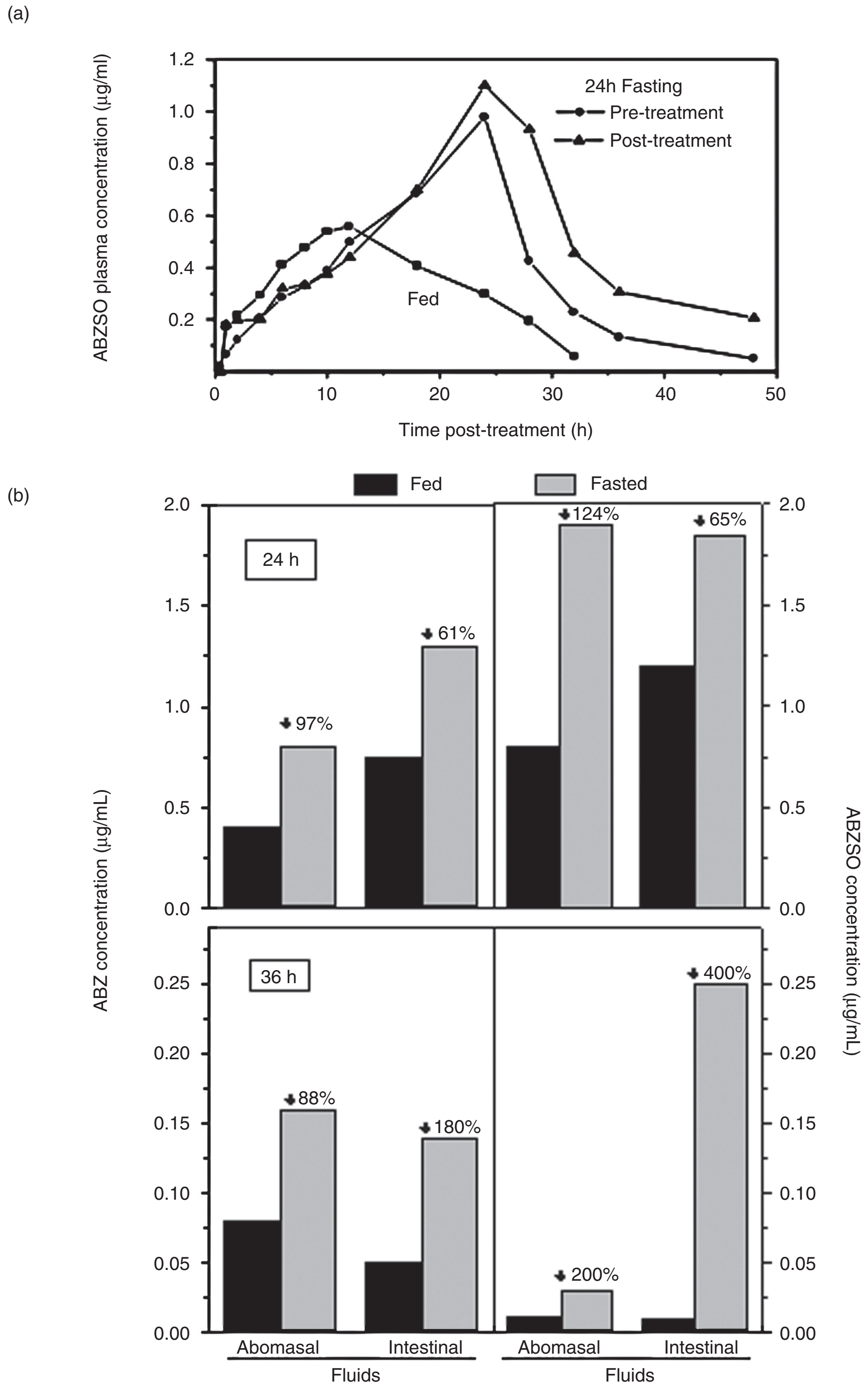


Figure 39.9 Influence of fasting on the kinetics of albendazole in cattle**.** (a) Mean albendazole sulfoxide (ABZSO) plasma concentrations obtained after the intraruminal administration of albendazole (ABZ) (10 mg/kg) to calves either fed *ad libitum* (control) or fasted during 24 h prior or after the treatment. The systemic availability of ABZSO increased between 96% (fasting pretreatment) and 118% (fasting posttreatment) in fasted compared to fed *ad libitum* calves. (b) Mean concentrations of ABZ (left panel) and ABZSO (right panel) obtained in abomasal and intestinal fluids at 24 and 36 h after the intraruminal administration of ABZ (10 mg/kg) to calves either fed *ad libitum* or fasted for 24 h pretreatment. The figures on the top of each bars represent the enhancement (percentage) on drug concentrations in the different digestive fluids observed in fasted compared to animals fed *ad libitum*. Source: (a) Adapted from Sánchez et al., 2000a. Reproduced with permission of Elsevier. (b) Sánchez et al., 2000b. Reproduced with permission of John Wiley & Sons.

both, the decreased energy (ATP) production and the reduced NADPH availability in the liver cell may have contributed to the decreased rate of albendazole biotransformation and delayed elimination of albendazole and its metabolites observed in calves subjected to undernutrition.

The presence of the parasite itself could induce important changes to the pharmacokinetics, side-effects, and expected efficacy of the chosen BZD anthelmintic for therapy. Liver disease and parasite-mediated liver damage with alteration of liver enzyme pattern could affect biotransformation and the resultant bioavailability of anthelmintically active BZD metabolites. A reduced enzymatic activity of different liver microsomal oxidases has been reported in *Fasciola hepatica*-infected sheep (Galtier et al., 1986b), which could lead to altered patterns of drug/xenobiotic metabolism and clearance. A significant decrease in the rate of both albendazole sulfonation and albendazole sulfone elimination has been demonstrated in sheep infected with *Fasciola hepatica* (Galtier et al., 1991). These findings correlated with a 58% decrease in the rate of albendazole sulfonation by liver microsomal preparations obtained from 8-weeks infected sheep; these authors postulated that a decrease in the liver microsomal cytochrome P-450-dependent monooxygenase activity, induced by the presence of the immature liver flukes on the hepatocyte parenchyma, could likely be the cause of such a metabolic change.

Parasite-mediated inflammatory reactions with changes in mucosal permeability and in abomasal/intestinal pHs, could have an impact both on absorption and ionic-trap-mediated distribution of different BZD molecules. Elevation in abomasal pH and increased permeability of the mucosa to macromolecules has been demonstrated during abomasal parasitism. The pathophysiological changes occurring during GI parasitism may affect the absorption of BZD compounds due to modifications in the GI transit time and to the atrophy of intestinal villi. Some studies on the influence of GI parasitism on the plasma kinetics of different anthelmintic drugs have been reported. Following artificial infection with abomasal nematodes (*Teladorsagia circumcincta*, *Haemonchus contortus*), the pattern of absorption and systemic availability of fenbendazole (Marriner et al., 1985), oxfendazole (Hennessy et al., 1993b), and febantel (Debackere et al., 1993) in sheep, have been shown to be altered. On the other hand, the presence of moderate nematode infections was sufficient to identify important changes to the plasma and abomasal disposition kinetics of albendazole in both artificial and naturally infected sheep (Alvarez et al., 1997). The main modification observed in infected sheep was the elevation of the abomasal pH. The increased abomasal pH induced by the presence of the parasite, decreased the plasma/abomasum pH gradient and reduced the ionic trapping of BZD molecules in the abomasum, which may have a relevant impact on anthelmintic efficacy.

Factors Related to Pharmaceutical Formulation

As introduced in Chapter 5, pharmaceutical formulation can have a major impact on drug disposition. This is particularly true for the antiparastitic drugs presented here. The relevance of water solubility and rates of dissolution of drug particles in the GI tract lumen on the absorption and resultant pharmacokinetic behavior of BZD compounds has been extensively demonstrated. Further evidence was obtained by altering the crystalline nature of the oxfendazole molecule. The production of an amorphous form of oxfendazole by acidic treatment resulted in a marked change in water solubility from 4 μg/ml (crystalline form) to 11.3 μg/ml (amorphous form) at a pH of 7.6. The oral administration of amorphous oxfendazole resulted in a more rapid and complete absorption compared with the crystalline form. It was shown that half the dose of the amorphous oxfendazole produced equivalent efficacy to that of the normal crystalline oxfendazole. Erratic absorption and variable systemic availability can be expected after administration of low-quality BZD suspensions, where large drug particles size and poor pharmacotechnical elaboration may affect the rate of dissolution and resultant absorption in the GI tract. In fact, marked differences in the albendazole sulfoxide systemic exposure have been observed among different commercially available “generic” albendazole formulations (Suarez et al., 2011), which has been associated with the impact of the pharmaceutical quality on the dissolution of albendazole particles and resultant GI absorption.

Different pharmaceutical strategies have been investigated to overcome the limited GI dissolution and absorption of BZD anthelmintics. An injectable formulation (solution) of albendazole sulfoxide (ricobendazole) has been developed for use in cattle, exploiting its greater hydrosolubility compared to other BZD methylcarbamates (Lanusse et al., 1998). Development of more water-soluble prodrugs, albendazole loaded nanoparticles (Rodrigues et al., 1995), aqueous formulation of albendazole with cyclodextrins (Evrard et al., 2002), and surfactant-based albendazole suspensions (Virkel et al., 2003) are among other strategies used to enhance the GI absorption of BZD compounds. A marked improvement in the systemic availability of albendazole metabolites has been reported after administration of an albendazole suspension prepared with sodium lauryl sulfate in cattle (Virkel et al., 2003).

Withdrawal Times, Safety, and Toxicity

As a consequence of tissue and milk residues of BZD compounds, slaughter clearance times are required after treatment with substituted BZD and it is recommended that milk of treated animals not be used for human consumption. Withdrawal times of 3 days (meat) and 4 days (milk) are required after thiabendazole treatment in cattle. When thiabendazole is used as a single oral dose in sheep or goats, 30 days withdrawal time to slaughter time is recommended. Withdrawal times are required after albendazole treatment in beef cattle (27 days) and sheep (7–10 days). For fenbendazole given as a suspension, a period of 8 days (cattle) and 6 days (goats) of withdrawal time is needed. However, since numerous commercial products containing BZD compounds are available around the world, it is critical to check label instructions of each formulation concerning withdrawal times before its use. BZDs are not indicated in horses to be slaughtered for food purposes. Flubendazole is widely used as an anthelmintic in poultry. Its main metabolites, hydrolyzed flubendazole and reduced flubendazole have been identified in tissues of flubendazole medicated turkeys (De Ruyck et al., 2001). Concentrations of flubendazole/metabolites in eggs collected from laying hen feed with 10 and 30 mg/kg of flubendazole reached a plateau level after 10 days. The residues of flubendazole and metabolites were detected mainly in yolk, where the main metabolite, accounting for almost 60–65% of the total residue, was hydrolyzed flubendazole (Kan et al., 1998). A 7-day withdrawal time is required for flubendazole in broiler chickens. Pigs may be slaughtered for human consumption after 7 days following flubendazole treatment.

BZDs are probably one of the least toxic of the available anthelmintics. The remarkable overall safety of BZD compounds has been a major factor in their successful worldwide use. All BZDs are extremely well tolerated by domestic animals and man, and they are usually free of side-effects at therapeutic doses, even when administered to young, sick, or debilitated animals. For instance, oxfendazole does not cause detectable toxic effects at 10 times the recommended dose or at eight successive administrations of three times the therapeutic dose at 4-day intervals in ruminants and horses. Similarly, animals treated with albendazole at five to nine times the therapeutic dose, did not show any adverse events after treatment (Alvarez et al., 2012). The high safety margins, particularly for the most potent substituted BZD, is thought to be correlated with their low solubility in the GI fluids; this account for a low absorption rate and for insufficient drug reaching the bloodstream to induce toxic effect. Some members of the group, such as parbendazole, cambendazole, oxfendazole, febantel and albendazole, have been reported to be teratogenic at approximately four times their recommended doses; this limits their use in the early stages of pregnancy and sheep seem to be particularly sensitive compared to other animal species (McKellar and Scott, 1990). The administration of oxfendazole or albendazole in pregnant cows caused no increase in the incidence of congenital abnormalities in the offspring. Similar results have been reported in pigs and horses. Teratogenic effects may occur at dosages much lower than those associated with acute toxicity in target species. The observed species differences in the sensitivity to the teratogenic activity may be related to differences in the pharmacokinetics and metabolism of BZD among species. The main malformations observed following treatment at day 20 of pregnancy have been deformities of the limbs and overflexion of the carpal joints. In ewes treated with NTB at the day 17 of pregnancy, congenital malformations (skeletal and renal) and abortions were observed (Navarro et al., 1998). The types of malformations induced by flubendazole in rats after its oral administration (160 mg/kg/day for 7 consecutive days between day 8 through 15 of pregnancy) are similar to that described for parbendazole and cambendazole, and include encephalocele, meningocele, hydrocephaly, short tail, absent tail, fusion or agenesis of vertebrae, and fused ribs. The mechanism of BZD teratogenicity seems to be related to their effects on the disruption of the tubulin–microtubules equilibrium dynamics in mammalian cells and subsequent alteration of cell division. Since the main toxic effects are teratogenic, some substituted BZD molecules are contraindicated to be used in early pregnancy. Albendazole is not indicated for use during the early pregnancy in cows (45 days) and ewes (30 days). The administration of febantel as tablets in dogs and cats is not recommended in pregnant animals. However, febantel and mebendazole do not appear to exert a teratogenic effect in sheep when administered in early pregnancy.

Large doses of thiabendazole (a more soluble BZD compound) administered for a long period has been associated with anemia in dogs. In-feed flubendazole treatment in poultry does not affect egg production, fertility, and hatchability. In spite of the antimicrotubular effect of BZD molecules, different experiments involving fenbendazole, albendazole, or oxfendazole in bulls, rams, or stallions did not demonstrated adverse effects on reproductive function including spermatogenesis, testicular weight, and testosterone production (McKellar and Scott, 1990).

Imidazothiazoles

Chemistry

The first imidazothiazole anthelmintic introduced into the veterinary market (1967) was tetramisole. It is a racemic mixture of two optical isomers in equal amounts: S(−)tetramisole (L-tetramisole or levamisole) and R(+)tetramisole (D-tetramisole). Following approval and marketing of the racemic mixture, pharmaceutical scientists were able to separate D L-tetramisole into its two isomers. Further studies discovered that the anthelmintic activity of the racemic mixture was attributable almost solely to the L-isomer. Thus, by using the L-isomer alone, the dosage could be reduced by half. This also increased the safety margin since both components of tetramisole are similarly toxic but differ in anthelmintic efficacy. Currently, levamisole is the worldwide imidazothiazole compound available for use in veterinary medicine. However, in some countries, tetramisole formulations are still available. The information described below is based on the pharmacological properties of levamisole, the most extensively used drug in this chemical class.

Levamisole is the L-isomer of tetramisole. The chemical name of levamisole is (−)-2,3,5,6-tetrahydro-6-phenylimidazo[2,1-*b*] thiazole (Figure 39.10). Levamisole is an antinematodal drug with a broad range of activity in several host species. It is worldwide approved and marketed for use in cattle, sheep, swine, poultry and dogs. Levamisole is a nematodicidal compound effective against lung and GI tract nematodes but not against cestode and trematode parasites. A major advantage is its formulation flexibility rendering optional routes of administration (oral, parenteral, topical). Depending upon formulation, the drug is marketed as levamisole hydrochloride or phosphate salts (oral drenches, feed premixes and injectable preparations) and as levamisole base (pour-on). Levamisole hydrochloride, a white to pale cream crystalline powder, is highly soluble in water (1 g in 2 ml of water). This solubility facilitates the formulation of an injectable solution and a stable drench.

Mode of Action

Levamisole is a cholinergic receptor agonist and elicits spastic muscle paralysis due to prolonged activation of the excitatory nicotinic acetylcholine receptors

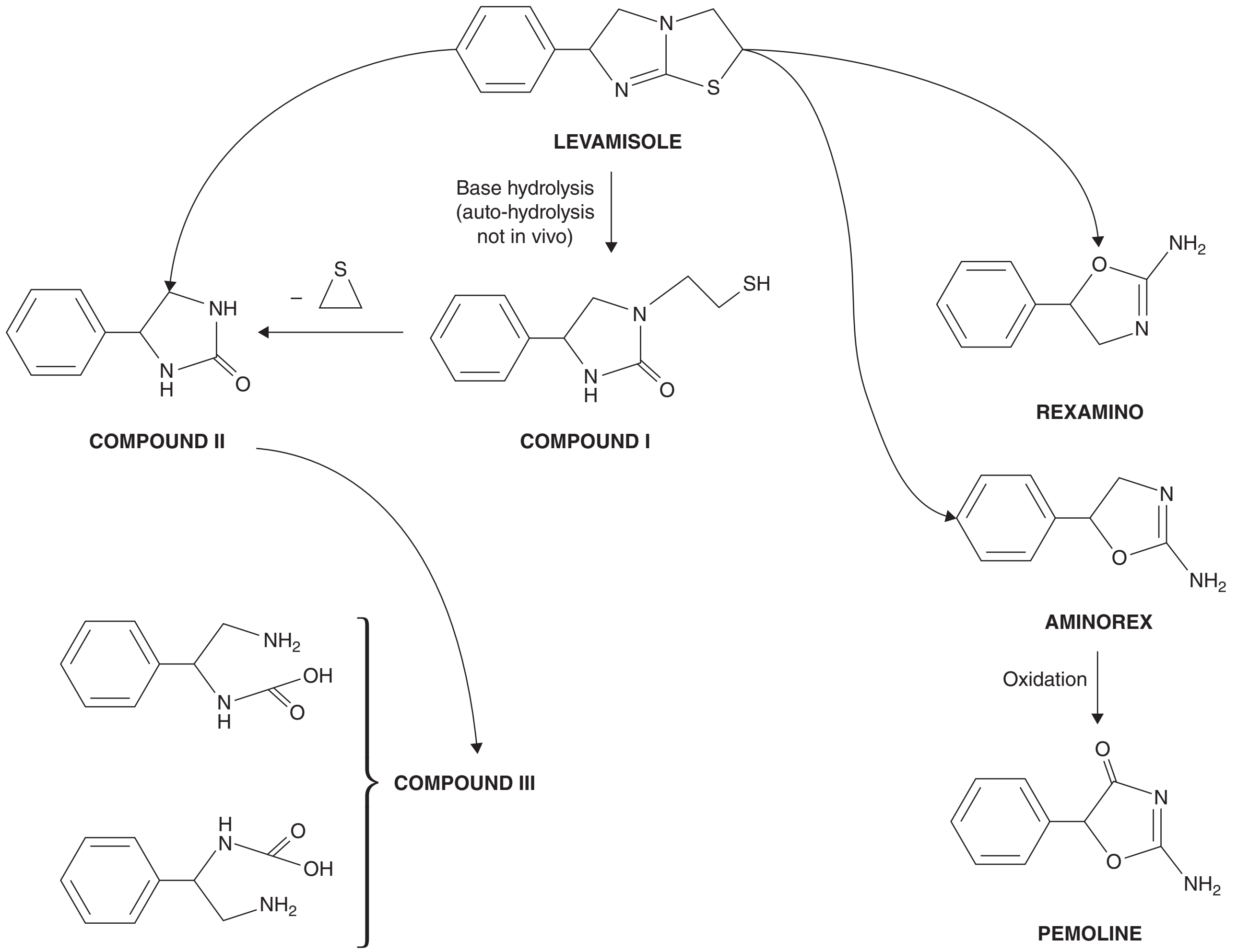


Figure 39.10 Proposed metabolic pathways of levamisole. Compound I is generated in vitro by a base hydrolysis that occurs spontaneously. Compound II, aminorex, and rexamino are formed from levamisole, while compound III and pemoline are secondary metabolites formed from compound II and aminorex, respectively.

(nAChR) on nematode body wall muscle. Levamisole's precise mode of action has been carefully studied at the single-channel level on body wall muscle preparation of *Ascaris suum* (Martin et al., 2004, 2005). Pharmacological analysis has provided evidence for subtypes of nAChR (Qian et al., 2006): an N-type (preferentially activated by nicotine and oxantel), a B-type (preferentially activated by bephenium), and an L-type (preferentially activated by levamisole and associated with levamisole resistance). Levamisole, and related compounds, also cause spastic paralysis and egg laying in *Caenorhabditis elegans*. Indeed, recordings from *Caenorhabditis elegans* body wall muscle using levamisole and nicotine as agonists have provided further evidence that there are muscle subtypes of nAChRs; these subtypes seems to have different nAChR subunit compositions. At least four subunits contribute to the levamisole receptor (Culetto et al., 2004; for further information on the nACh receptor subunit family see Rand, 2007). Its uptake in helminth parasites is considered to be mainly by a transcuticular mechanism.

Pharmacokinetics

The rate of levamisole absorption differs with the route of administration. The drug is most rapidly absorbed following intramuscular or subcutaneous injection in cattle and the highest plasma levels (>1 μg/ml) are observed at 0.5–2 hours. Several oral formulations gave similar absorption rates (T_{max}= 3 hours) and slower absorption was observed after dermal application (Bogan et al., 1982). Bioavailability differs depending on the route of administration. In sheep, the highest mean plasma concentrations were achieved after subcutaneous (3.1 μg/ml) compared to oral (0.7 μg/ml) and intraruminal (0.8 μg/ml) administration at a dose rate of 7.5 mg/kg (Bogan et al., 1982). Following pour-on administration in cattle, plasma and GI concentrations of levamisole were lower than those measured following parenteral and oral treatments (Forsyth et al., 1983), which agrees with the limited anthelmintic efficacy reported for the topical preparation. An oral bioavailability of 49% has been demonstrated in dogs, which increased to 64% when animals were fasted for a total period of 24 hours (Watson et al., 1988). In small ruminants, levamisole systemic availability has been shown to be 25–33% higher after parenteral administration compared to the oral route (Fernández et al., 1998; Sahagun et al., 2000; 2001). It has been reported (El-Kholy et al., 2006) that levamisole bioavailability in laying hens is higher at the peak egg production (88%) compared to prelaying hens (61%). Also, higher volume of distribution at steady-state (Vd_{ss}) (8–13 l/kg) and whole-body clearance (Cl_B) (2.5–3 l/kg/h) values were observed (El-Kholy et al., 2006). Garcia et al. (1994) reported that absorption was faster after the subcutaneous (T_{max} = 17 minutes) compared to the oral administration (1.12 hours) of levamisole in rabbits. The bioavailability was almost complete after subcutaneous administration, while a lower value (60%) was observed after oral administration, similar to that reported for dogs (Garcia et al., 1994). Absorption and systemic bioavailability are important because, given the mode of anthelmintic action of levamisole, the level of concentration is more important than the time of parasite exposure to the drug (Lanusse and Prichard, 1993a).

Once systemically available, levamisole is widely distributed in the organism (Vd_{ss} of 3.8, 2.75, and 2.13 l/kg in rabbits, goats, and sheep, respectively), and is recovered in tissues such as muscle, fat, kidney, and, particularly, liver at 2 hours postadministration of an oral or subcutaneous dose. In cattle, the highest milk concentrations are achieved at 1 hour postadministration. Levamisole plasma concentrations decline over a period of 6 to 8 hours, with 90% of the total dosage being excreted in 24 hours. Levamisole is rapidly and extensively metabolized to a large number of metabolites in the liver. The main metabolizing pathways appear to be oxidation, hydrolysis, and hydroxylation. Oxidation of the imidazothiazole ring is followed by oxidation to a carbonyl and hydrolysis to a thiohydantoic acid. Excretion of both levamisole and metabolites (glucuronyl or *S*-cysteinyl-glycine conjugates) is mainly in the urine (about 60%) and feces (about 30%). Unchanged levamisole accounted for 5–10% of the dose in urine and feces in cattle, sheep, and swine (Nielsen and Rasmussen, 1982; IPCS, 2003). In horses, levamisole metabolism has been well characterized because its administration to race horses led to the identification of trace amounts of prohibited substances in urine (Barker, 2009; Gutierrez et al., 2010). After oral and subcutaneous administrations to horses, levamisole is metabolized by the liver to four main metabolites (Figure 39.10): compound II, aminorex, rexamino (isomer of aminorex), and pemoline (oxidized form of aminorex) (Ho et al., 2009; Gutierrez et al., 2010). Compound II is further metabolized to the isomeric compound III (Ho et al., 2009). Plasma persistence is longer for aminorex (36 hours), rexamino, and compound II (72 hours) compared to levamisole (12 hours) (Ho et al., 2009). These metabolites are mainly excreted by the urine, where free and sulfates or glucuronide conjugated forms of unchanged and hydroxylated levamisole are also found. Also, four hydroxyl metabolites of aminorex were described (Hess et al., 2013). Aminorex is an amphetamine-like agent. Since it was found to cause pronounced vasoconstriction in the pulmonary vasculature, it was withdrawn due to cases of fatal and life-threatening pulmonary hypertension (Fishman, 1999). Pemoline is a central nervous system stimulant (Gutierrez et al., 2010).

The reported elimination half-life values are: 4–6 hours (cattle), 1.8–4 hours (dogs), 3.5–6.8 hours (pigs), 2 hours (horses), and 0.96 hours (rabbit). Levamisole is fast depleted from the animal body and tissue residues of the drug are not appreciable. Approximately 0.9% of the initial dose is found in tissues (liver and kidney) at 12–24 hours after dosing. By 7 days after dosing, levamisole is not detectable in muscle, liver, kidney, fat, blood, or urine of rats or other animals tested. Unchanged levamisole accounts only for 3% of the total tissue residues measured over a 14-day period.

Anthelmintic Spectrum

The compound has a broad spectrum of activity against mature stages of the major GI nematodes and against both mature and larval stages of lungworms. However, levamisole shows little action against arrested larval stages. Depending on the product licensed, levamisole is indicated for nematodes in cattle, sheep, goats, swine, poultry, dogs, and cats. In ruminants, levamisole has relatively good activity against abomasal nematodes, small intestinal nematodes (not particularly good against *Strongyloides* spp.), large intestinal nematodes (not *Trichuris* spp.), and lungworms. Adult stages of the nematodes that are usually covered by levamisole include: *Haemonchus* spp., *Ostertagia* spp., *Cooperia* spp., *Nematodirus* spp., *Bunostomum* spp., *Oesophagostomum* spp., *Chabertia* spp., and *Dictyocaulus vivaparus*. Levamisole is less effective against the immature forms of these parasites and is generally ineffective in cattle against arrested larval forms. In swine, levamisole is indicated for the treatment of *Ascaris suum*, *Oesophagostomum* spp., *Strongyloides ransomi, Stephanurus dentatus*, and *Metastrongylus* spp. Levamisole has been used in dogs against GI nematodes and as a microfilaricide to treat *Dirofilaria immitis* infection at a dosage of 5.5 mg/kg twice daily (12-hour interval) for 6 days (or up to 15 days for persistent microfilaremias). Because of its narrow safety margin and limited efficacy against many equine parasites, levamisole is not generally used in horses.

Formulations, Dosages, and Routes of Administration

Levamisole is administered as tablet, solution, oral drench, feed additive, subcutaneous injectable solution, or topical pour-on. In cattle, sheep, goats, and pigs, a dose of 8 mg/kg levamisole is recommended in a single oral or subcutaneous administration and 10 mg/kg as a pour-on for cattle. The bioavailability of levamisole in sheep was significantly lower after oral (42%) and intraruminal (45%) administrations compared to the injectable treatment (Bogan et al., 1982). Administration of levamisole in drinking water is used routinely only for swine and poultry. For poultry, the medicated drinking water is prepared by calculating the total amount of drug needed to provide a dose of 40 mg/kg. As the peak concentration rather than the duration of exposure is important for the anthelmintic effect of levamisole, medicated feed should be consumed fairly rapidly, otherwise efficacy may be reduced.

Injectable levamisole is preferred for use in cattle. The original, aqueous solution of levamisole hydrochloride was administered by intramuscular or subcutaneous injection. The hydrochloride, however, proved to be irritating to tissues, and intramuscular injection resulted in moderate to severe reactions at the injection site. The monobasic phosphoric acid salt of levamisole was found to be less irritating to tissues. Pour-on formulation for cattle contains 10–20% levamisole as base, which is absorbed through the skin following application to the midline of the back.

Levamisole is approved for use in dogs and cats in some countries. Tablets and oral solutions are used in dogs and cats at dose rates between 5 and 10 mg/kg. Oral treatment with 10 mg tetramisole/kg/day for 2 days removed more than 95% of ascarids (*Toxocara*, *Toxascaris*) and hookworms (*Ancylostoma*, *Uncinaria*). Levamisole is not effective against canine whipworms (*Trichuris vulpis*). Overall, levamisole remains a useful nematodicidal compound as a result of an acceptable margin of safety and spectrum of activity coupled with very low cost.

Immunomodulatory Effects

In addition to its anthelmintic activity, levamisole also may enhance immune responsiveness. This characteristic has caused considerable excitement in both veterinary and human medicine. Levamisole has proved to act as an immunomodulator in experimental models and in a number of selected human and animal immunodeficiencies with related pathology. These properties have been extensively reviewed (Koller, 1982; Blecha, 1988). The studies on its application in veterinary practice, mainly in bovine practice, have been also reviewed (Desplenter, 1983). Levamisole modulates the immune function at 2–3 mg/kg of body weight, in contrast to the greater anthelmintic doses. When administered at higher doses, levamisole may even suppress immune function. Intermittent treatment is more efficient than continuous treatment in restoring immune responsiveness. In vitro and in vivo, levamisole is able to restore to normal the major functions of effector cells of the cell-mediated immune response. This restoration is most pronounced and consistent in compromised hosts whose T-lymphocytes or phagocyte functions are below normal. Usually, an adequate immune response is not increased. The B-lymphocyte activity is not directly stimulated; the proliferative response to mitogens is not increased and there is no direct effect on antibody production. However, it should be kept in mind that the responses to levamisole are not always predictable, even if it is used in appropriate conditions. The results fluctuate between highly favorable and no effect. In cattle, for example, protective effects have been obtained in the prevention or reduction of complications associated with shipping fever (Desplenter, 1983).

Safety and Toxicity

The pharmacodynamic actions of levamisole (or tetramisole) in the host suggest that the drug exerts both muscarinic and nicotinic effects. Signs of levamisole intoxication (salivation, defecation, and respiratory distress from smooth muscle contraction) are like those observed during organophosphate poisoning. Indeed, evidence suggests that some of the toxicity of this drug may be concerned with cholinesterase inhibition, leading to manifestations of the muscarinic action of acetylcholine (ACh) (i.e., constriction of pupils and respiratory bronchioles, acceleration of GI motility, slowing of the heart rate, and other autonomic actions).

Tetramisole itself has a safety margin estimated to be two to six times the therapeutic dose of 15 mg/kg. Tetramisole is lethal to sheep at a dose of 90 mg/kg. At a dose of 45 mg/kg signs of toxicity occur. Side effects or death are more likely to occur when tetramisole is administered parenterally. The safety factor of levamisole is about twice that of the parent tetramisole compound. Levamisole is considered to be more dangerous when administered parenterally than oral or topical treatments. Intravenous administration is particularly hazardous and is never recommended. Cattle appear to be somewhat more tolerant of parenteral administration than sheep. A twofold overdose of injectable levamisole phosphate may cause about two-thirds of treated cattle to lick their lips and to develop temporary foaming of the muzzle. The pour-on formulation has been tested on several thousand cattle with only occasional dermal irritation. When given to pigs at three times the recommended dosage, levamisole causes only occasional vomiting. In such cases, the reaction is due to expulsion of the worms and should terminate in several hours. The LD_{50} of subcutaneously injected levamisole in pigs is 40 mg/kg. Simultaneous administration of pyrantel tartrate (a nicotine-like drug) enhances toxicity by lowering the levamisole LD_{50} value to 27.5 mg/kg.

Chickens tolerate tetramisole and levamisole very well. The LD_{50} for chickens is quite high (2.75 g tetramisole/kg), and minimum toxic levels of the drug in chickens exceed 640 mg/kg. In geese, however, a dosage of 300 mg/kg is known to be toxic. In chickens, therapeutic doses of levamisole (36–40 mg/kg) cause no undesirable side effects, and egg production, fertility, and hatchability are not affected adversely. Dogs and cats are much more tolerant to oral than parenteral administration of tetramisole. When given orally to dogs, doses of 20 mg/kg are well tolerated, and even 40 and 80 mg/kg are not fatal, although vomiting occurs. Subcutaneous tetramisole at 40 mg/kg is fatal to dogs in 10–15 minutes; even at 20 mg/kg, the drug causes severe reactions in dogs, although they persist for only about 20 minutes. On the basis of these findings, the oral route of administration is recommended for dogs and cats. Imidazothiazole compounds have a narrow margin of safety in horses and they are not approved for use in this species.

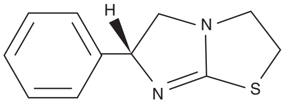
Levamisole should be administered within specified time periods before slaughtering swine, cattle, or sheep. A 2 to 11-day slaughter clearance time is mandated, depending on the formulation used and animal species. The identified levamisole metabolites are much less toxic than the parent compound, so the parent drug is sought in analysis of tissue samples. Since no withdrawal time has been established for dairy cows, this drug should not be administered to lactating cattle producing milk for human consumption. There are no specific contraindications in administration of levamisole with other drugs. However, other nicotine-like (e.g., pyrantel, morantel) or cholinesterase-inhibitor drugs (e.g., organophosphate, neostigmine) could theoretically enhance the toxic effects of levamisole.

Tetrahydropyrimidines

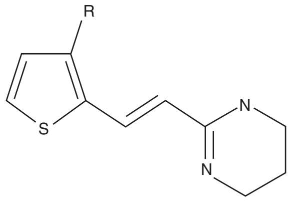
Pyrantel, introduced in 1966, was the first compound within the tetrahydropyrimidines family. It was initially used as a broad-spectrum anthelmintic against GI nematodes in sheep, and thereafter developed for use in cattle, swine, horses, dogs, and cats. Subsequently, its methyl ester analogue, morantel, was also introduced as a nematodicidal compound in the veterinary market (Figure 39.11). The newest drug in this chemical family is oxantel, an m-oxyphenol derivative of pyrantel. However, due to its cost, it is not very used in veterinary medicine.

Chemistry

Pyrantel is E-1,4,5,6-tetrahydro-1-methyl-2[2-(2-thienyl)vynil]-pyrimidin (Figure 39.11). It is formulated as tartrate, citrate, or pamoate (also known as embonate) salts. Pyrantel pamoate is practically insoluble in water and alcohol, while the tartrate salt is more water soluble. Each gram of pyrantel pamoate is approximately equivalent to 347 mg (34.7%) of the base. Pyrantel citrate is equivalent to 410 mg (41%) of the base. Pyrantel salts are relatively stable in the solid phase. Aqueous solutions are subject to photoisomerization upon exposure to light, with losing of potency. Morantel (1,4,5,6-tetrahydro-1-methyl-2-[2-(3methyl-2-thienyl)ethenyl]pyrimidine)



S(–) LEVAMISOLE



PYRANTEL; R = H
MORANTEL; R = CH_3

Nicotinic receptor agonist	MODE OF ACTION	Nicotinic receptor agonist
Lung and GI nematodes (mainly adult stages)	ANTHELMINTIC SPECTRUM	GI nematodes (narrow, adult stages)
Side-resistance with pyrantel and morantel	ANTHELMINTIC RESISTANCE	Side-resistance with levamisole
Rapid absorption (injectable) Limited absorption (oral, topical) Fast elimination	PHARMACOKINETICS	Poor GI absorption (ruminants) Good GI absorption (dog, pig)
Subcutaneous, oral, topical	ROUTES OF ADMINISTRATION	Oral (all species) Slow release bolus (cattle)
Hydrochloride, phosphate	FORMULATIONS	Pamoate, tartrate
Narrow safety margin (caution in horses)	TOXICITY	Good safety margint

Figure 39.11 Chemical structures and some comparative pharmacological properties of the imidazothiazole (levamisole) and tetrahydropyrimidine (pyrantel, morantel) anthelmintic compounds. GI, gastrointestinal.

is mainly formulated as a tartrate salt (Figure 39.11). Each gram of the tartrate salt is equivalent to 59.5% of base activity. Oxantel is (1-methyl-2-(3-hydroxyphenyl-ethenyl)1,4,5,6-etrahydropyrimidine.

Mode of Action

Tetrahydropyrimidine compounds act selectively as agonists at synaptic and extrasynaptic nicotinic acetylcholine receptors (nAChR) on nematode muscle cells and produce contraction and spastic paralysis. As explained above (see Section Levamisole, Mode of Action), there are three different types of nAChR: L, N, and B type. Pyrantel, as levamisole, is an L-type receptor agonist, while oxantel is an N-type receptor agonist (Martin et al., 2004, 2005; Qian et al., 2006). Pyrantel and morantel are 100 times more potent than acetylcholine, although slower in initiating contraction. Pharmacological effects of pyrantel, oxantel, and morantel on the host are similar to the effects of levamisole. These anthelmintics share biological properties with acetylcholine and act essentially by mimicking the paralytic effects of excessive amounts of this natural neurotransmitter. Pyrantel, oxantel, morantel, levamisole, and diethylcarbamazine mimic this paralytic action, which is similar to the paralytic effect caused by nicotine; thus the action of these anthelmintics is referred to as nicotine-like effect. Although the chemical structures of oxantel and pyrantel are similar, oxantel offers some advantages in efficacy against *Trichuris* spp., which are due to differences in the nAChR selectivity between the molecules as well as to a difference in the dominant cholinergic receptor subtype present in these parasites compared to other intestinal nematodes (Martin et al., 2004; Kopp at al., 2009; Holden-Dye et al., 2013).

Pharmacokinetics

The pyrantel pamoate salt is poorly absorbed from the GI tract and high concentrations of the unabsorbed drug reach the lower digestive tract in dogs, cats, and horses. Pyrantel tartrate is absorbed more readily than the pamoate salt. The tartrate salt is better absorbed in pigs and dogs compared to ruminants, with peak plasma levels occurring 3–6 hours after its oral administration. Peak plasma levels occur at highly variable times in ruminants. Absorbed drug is rapidly metabolized and excreted into the feces. Pyrantel is extensively metabolized in dogs, rats, sheep, and cattle by oxidation of the thiophene ring, oxidation of the tetrapyrimidine ring, and mercapturic acid conjugation. From the in vivo radiolabel studies, it was shown that the thiophene ring underwent extensive degradation leading to acidic metabolites that are highly polar, being eliminated mainly by urine. Urinary excretion of pyrantel accounts for about 40% of the dose in the dog and 34% in the pig, about 80% of which is excreted as metabolites. The dog is the only species excreting a larger proportion of the drug/metabolites in urine compared to feces. In ruminants, urinary excretion accounts for about 25% of the original dose, much of the remainder passing unchanged in feces. In rats, urinary excretion of the drug is minor; bile is the major route of excretion of metabolites of the absorbed drug.

Morantel is negligibly absorbed in ruminants. The drug is undetectable in the bloodstream in cattle after oral treatment. Morantel is largely excreted as the unmetabolized parent compound in the feces. In a pharmacokinetic and GI compartmental distribution study in calves treated with a morantel tartrate slow-release bolus (Lanusse et al., 1992a), it was shown that the morantel peak concentration is reached at 24 hours postadministration in all GI compartments and in feces. A steady-state concentration was reached at 7–10 days posttreatment and maintained over 84–91 days in feces and in ruminal fluid, and over 98 days in abomasal and ileal fluids. These concentrations of morantel in the GI tract of cattle may account for removal of established worms with the initial high peak concentration, and prevention of establishment of incoming larvae with a plateau steady-state concentration, which is desirable to minimize selection for drug resistance.

Formulations, Dosages, and Routes of Administration

Both the tartrate and pamoate salts of pyrantel are used for parasite control in horses. Pyrantel tartrate is formulated for continuous, daily administration over prolonged periods of parasite exposure. Pyrantel pamoate can be administered in suspension or paste formulations, as well as by mixing with feed. Regardless of the method of administration, a dose of 6.6 mg pyrantel base/kg should be used. A powdered premix formulation containing 10.6% pyrantel tartrate is available for treating parasitic infections of swine via medicated feed. Overnight fasting is advised. Water should be available ad libitum during the fasting and treatment periods.

Formulations of pyrantel pamoate for dogs include suspension and tablet forms. Pyrantel pamoate is also combined with febantel and praziquantel in a tablet form for dogs. Each of the above formulations is given to dogs as a single dose. A higher dose of pyrantel pamoate (20 mg/kg) is combined with praziquantel (5 mg/kg) for cats. Administration with food delays passage through the digestive tract, prolongs contact time of the drug with parasites, and thereby increases efficacy. Mackenstedt et al. (1993) proved that preadult stages of *Toxocara canis* continuously absorbed the drug through the whole body surface and that duration of exposure to pyrantel is more important in efficacy than variations in dosage. Other formulation for dogs is a beef-based chewable form that combines ivermectin (6–12 μg/kg) for control of heartworms and pyrantel pamoate (5–10 mg/kg) for control of hookworms and ascarids. This form is administered once a month.

A sustained-release bolus of morantel tartrate for both dairy and beef cattle is widely used in Europe and has been approved in many countries in America. The drug (11.8 g morantel base) is packaged in a cylindrical trilaminate cartridge, which is administered orally by a special delivery device and is retained in the rumen/reticulum. This devise is known as the MSRT (morantel slow release trilaminate bolus). The permeable wall allows continuous release of morantel tartrate (approximately 150 mg/day) into the rumen/reticulum fluid for at least 90 days (Lanusse et al., 1992a). Administration of the MSRT bolus is recommended as preventative for larvae establishing as well as to control existing adult nematode burdens in cattle. The ultimate effect is marked reduction in pasture contamination for a prolonged period, for example 90 days of drug release and benefits that extend for another 90 days. Inhibited *Ostertagia* larvae are not killed by this method of treatment, but lowered pasture contamination helps prevent development of type II ostertagiasis.

Dosages for pyrantel tartrate are: single therapeutic dose of 12.5 mg/kg (horses), 22 mg/kg (swine), or 25 mg/kg (sheep, cattle, goats). Dosages for pyrantel pamoate are: 6.6 mg base/kg (horses), suspension and chewable form at 5 mg base/kg (dogs); tablets at 5 mg/kg for dogs over 2.2 kg but 10–15 mg/kg for dogs less than 2.2 kg body weight. Morantel tartrate is recommended for oral treatment at 8.8–9.6 mg/kg (cattle) and at 10 mg/kg (sheep).

Anthelmintic Spectrum

In general, activity of pyrantel on GI helminths in horses is independent of the method of administration or the salt, that is tartrate or pamoate. Activity of each of the two salts is characterized by consistently high efficacies. A more limited efficacy has been observed against *Strongylus edentatus*, small strongyles, and mature and immature *Oxyuris equi.* Pyrantel is >95% effective against the ileocecal tapeworm (*Anoplocephala perfoliata*) at double the regular therapeutic dosage (i.e., 13.2 mg/kg) (Reinemeyer et al., 2006).

Pyrantel is indicated for the removal or prevention of the large roundworms (*Ascaris suum*) and *Oesophagostomum* spp. in swine. They also have activity against the swine stomach worm (*Hyostrongylus rubidus*). The citrate salt of pyrantel has similar efficacy to that of pyrantel tartrate in swine. Pyrantel is used to control the following parasites in dogs: ascarids (*Toxocara cani, Toxascaris leonina*), hookworms (*Ancylostoma caninum*, *Uncinaria stenocephala*), and stomach worm (*Physalopter*). It is useful for similar parasites in cats and is considered to be safe to use, even in kittens.

Pyrantel tartrate is an effective nematodicidal in ruminants. It is effective in sheep, cattle, and goats against GI nematodes such as *Ostertagia* spp., *Haemonchus* spp., *Trichostrongylus* spp., *Nematodirus* spp., *Chabertia* spp., *Cooperia* spp., and *Oesophagostomum* spp. The drug is highly effective against mature worms and any immature stages that dwell in the lumen. Morantel is an effective nematodicidal drug for use in sheep and cattle. The salts of morantel have greater anthelmintic activity than the parent compound, pyrantel. Efficacy of morantel tartrate is quite good against adult and immature stages of *Haemonchus* spp., *Ostertagia* spp., *Trichostrongylus* spp., *Cooperia* spp., and *Nematodirus* spp. The MSRT (morantel bolus) has been designed for removal of established worms (initial peak concentration) and prevention of establishment of incoming larvae (plateau steady-state concentrations).

Safety and Toxicity

The salts of pyrantel are free of toxic effects in all animal hosts at doses up to approximately seven times the therapeutic dose. The oral LD_{50} of pyrantel tartrate is 175 mg/kg in mice and 170 mg/kg in rats. In dogs, the acute oral LD_{50} for pyrantel pamoate is greater than 690 mg/kg (138 times the therapeutic dose). In chronic toxicity studies, dogs showed ill effects when administered pyrantel tartrate at 50 or more mg/kg/day for 3 months but no adverse effects when the dosage was reduced to 20 mg/kg/day for the same period. Pyrantel is safe for horses and ponies of all ages, including sucklings, weanlings, pregnant mares, and stallions. At 20 times the recommended dose in horses, ponies and foals, pyrantel pamoate shows no adverse clinical effects or changes in blood cell values or serum chemistry parameters. Pyrantel tartrate is slightly less tolerated in horses than the pamoate salt. The tartrate salt (100 mg/kg) produced death in one of three horses. Toxic signs preceding death included a marked increase in respiration rate, profuse sweating, and incoordination. No signs of toxicosis occurred following administration of 75 mg/kg. Ataxia is seen in some cattle treated with a high dose of pyrantel tartrate (200 mg/kg). The toxic dose of the drug in pigs is not known. Pyrantel is not recommended for use in severely debilitated animals, presumably because its pharmacological action (cholinergic) may be more pronounced in these hosts.

Withdrawal periods exist for swine and ruminants designated for slaughter. Because of lack of metabolism data in horses, the drug should not be used in horses intended for human consumption. Despite its cholinergic properties, there is no clinical evidence that simultaneous use of organophosphates increases toxicity. Thus labeling for pyrantel products indicates safety for simultaneous use with insecticides, tranquilizers, muscle relaxants, and central nervous system depressants. Pyrantel is recommended not to be used concurrently with other cholinergic agonist anthelmintics (morantel or levamisole). Piperazine and pyrantel/morantel have antagonistic mechanisms of action and their combined use should be avoided.

Morantel tartrate is a safer drug than pyrantel tartrate. The oral LD_{50} of pyrantel for mice is only 170 mg/kg while that of morantel is 5 g/kg. Chronic toxicity studies indicate that doses up to four times the therapeutic dose for sheep for 60 days and 2.5 times that for cattle for 20 days produce no toxic signs. Following a single therapeutic dose (10 mg/kg) via medicated feed, the drug is barely detectable (<0.05 mg/mL) in plasma or milk of lactating cattle and goats. Negligible levels of morantel tartrate in plasma and milk following single or sustained administration allow its use in dairy animals without a milk withdrawal restriction.

Organophosphate Compounds

Organophosphate compounds had their origins as pesticides and only subsequently found use as narrow-spectrum anthelmintics. Among the organosphosphates used as anthelmintics in domestic animals are dichlorvos, trichlorfon, haloxon, naphtalophos, coumaphos, naphthalophos, and crufomate. The first two were used primarily in horses, and the latter five in ruminants. Organophosphate compounds generally remove the principal parasites of horses, pigs, and dogs but are somewhat deficient in their activity against nematodes of ruminants. Dichlorvos remains useful to control nematode parasites in monogastrics and its spectrum includes bot flies (*Gasterophilus* spp.) in horses. Overall, the organophosphates have satisfactory efficacy for nematode parasites of the abomasum (especially *Haemonchus*) and small intestine but lack satisfactory efficacy for nematodes of the large intestine (*Oesophagostomum, Chabertia*). Other more efficacious and safer antiparasitic drugs have replaced them in the last 30–35 years. However, the current widespread development of resistance to the most modern anthelmintic families has motivated the use of some organophosphate compounds as the unique alternative to control multiresistant *Haemonchus contortus*. Additional information on the pharmacological properties of the organophosphate compounds can be obtained in Chapters 8 and 43.

Heterocyclic Compounds

Phenothiazine

Phenothizaine was perhaps the first anthelmintic to demonstrate a fairly wide range of activity against GI nematodes. Phenothiazine was used extensively to control nematodes in sheep, cattle, goats, horses, and chickens since 1938. Toxicity limited its use in swine and precluded its use in dogs, cats, and humans. As stated by Reinemeyer and Courtney (2001), the emergence of phenothizaine-resistant strains of ruminant and equine nematodes in the 1960s and competition from other broad-spectrum drugs markedly reduced the utility of this compound in subsequent years. It is no longer used as an anthelmintic compound. However, readers interested on specific information on phenothiazine are referred to the previous editions of this textbook, including the 8th edition (Reinemeyer and Courtney, 2001).

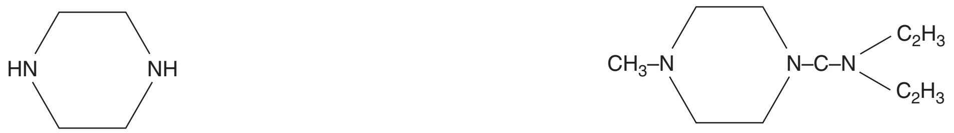
Piperazine (Diethylenediamine)

Discovered around 1900, piperazine was recognized as an anthelmintic moiety in 1954. It has good efficacy profiles against ascarid and nodular worm infections of all species of domestic animals, moderate for pinworm infections, and zero to variable for other veterinary helminths. Its low cost and wide safety margin in all domestic animals have led to its extensive worldwide use in antiparasitic therapy. Piperazine (MW 86.14 KDa) has a relatively simple chemical structure (Figure 39.12). It is a strong base, soluble in water (1 : 18), glycerol, and glycols, but only sparingly soluble in alcohol and totally insoluble in ether (Pharmaceutical Society of Great Britain, 1979). Piperazine is relatively unstable as the free base, and to improve its stability it is usually formulated as different salts such as adipate, citrate, phosphate, hexahydrate, and sulfate. Anthelmintic activity is directly related to the proportion of free base and this varies according to the salt form (e.g., adipate, 37% free base; chloride, 48%; citrate, 35%; dihydrochloride, 50–53%; hexahydrate, 44%; phosphate, 42%; sulfate, 46%)(Courtney and Roberson, 1995). Most piperazine salts are white crystalline powders that are readily soluble in water. Exceptions are adipate, which only dissolves to a maximum concentration of 5% in water, and phosphate, which is insoluble.

Piperazine is classified as a narrow-spectrum antinematodal drug since its efficacy has been only demonstrated against some specific nematodes in horses, pigs, dogs, cats, and chicken. In ruminants, this moiety is seldom used since its low efficacy against the commonest sheep abomasal parasites (*Teladorsagia circumcinta*) and other parasites located in the ruminants' small intestine. Piperazine's mode of action was initially thought to involve antagonism of cholinergic receptors located on the neuromuscular membrane. However, it is now clear that piperazine blocks transmission by hyperpolarizing nerve membranes at the neuromuscular junction leading to parasite immobilization by flaccid paralysis and consequent removal from predilection site and death. The most recent studies (Martin, 1997; Harder, 2000) demonstrate that piperazine is a selective agonist of γ-amino butyric acid (GABA) receptors, resulting in the opening of chloride channels and hyperpolarization of the membrane of the muscle cells of the nematode parasites. Mature worms are more susceptible to the action of piperazine than younger stages. Immature adults and lumen-dwelling larvae are sufficiently susceptible to be at least partially eliminated. Larval stages in host tissues, however, are relatively insusceptible. Because of subsequent larval development, repeated treatments are generally indicated within 2 weeks for carnivores and within 4 weeks for swine and horses.

Piperazine is readily absorbed through the GI tract and then extensively metabolized (60–70%). The remaining parent molecule is eliminated in urine over the 24-hour period following dosing. Piperazine base is detectable in urine as early as 30 minutes after the drug is administered. The therapeutic dose recommended for dogs and cats is 45–65 mg/kg of piperazine free base, given as a single oral administration. Efficacy has been demonstrated against *Toxocara* and *Toxoascaris*, although piperazine is ineffective against *Trichuris vulpis* (Jacobs, 1987). Treatment of nursing pups with piperazine at 2, 4, 6, and 8 weeks of age is highly effective (over 90%) in removing prenatally acquired *Toxocara canis* infections.

Dose recommended in horses, swine, and ruminants is 110 mg/kg. In horses, different piperazine salts are highly effective against ascarids, cyathostomes, and mature pinworms (*Oxyuris equi*). In foals, treatment at 8 weeks intervals is recommended for prevention of patent *Parascaris equorum* populations. Retreatment within 3–4 weeks is recommended in *Oxyuris equi* infections since piperazine has not demonstrated efficacy against immature pinworms. Single doses of piperazine are also effective (approximately 100%) against ascarids and nodular worms in swine. Retreatment 1–2 months later may



Piperazine (MW 86.1 Kda)		Diethylcarbamazine (MW 391.4 KDa)
GABA agonism, Flaccid paralysis	MODE OF ACTION	Inhibits cyclic peroxide generation from arachidonic acid breakdown
Narrow nematocidal (mainly ascarids)	ANTHELMINTIC SPECTRUM	Only microfilaria in dogs
Horses, dogs, cats, pigs, poultry	TARGET SPECIES	Dogs
Formulated as various salts Dogs, cats; 45–65 mg/kg Horses, pigs; 110 mg/kg Chicken; 32 mg/kg	FORMULATIONS DOSES	Tablets (6.6 mg/kg) Powder (2.5 mg/kg)
Narrow safety margin (caution in horses)	TOXICITY	Good safety margin

Figure 39.12 Chemical structures and some comparative pharmacological properties of piperazine and diethylcarbamazine.

be put into practice for optimizing the efficacy against migrating larvae from tissues after the first treatment. The drug is also recommended (two doses of 32 mg/kg every 24 hours) in two successive feedings (adipate of citrate) or drinking water (hexahydrate) as a highly effective treatment against *Ascaridia galli* in poultry. However, this treatment is apparently not effective against *Heterakis gallinarum*.

Experience over many years has confirmed the safety of piperazine; it is almost nontoxic under ordinary circumstances showing a large safety margin. However, large oral doses of piperazine produce emesis, diarrhea, incoordination, and head pressing in cats and dogs. In cats, piperazine was the anthelmintic for which toxicity (confirmed or suspected) was most frequently recorded by the *Illinois Animal Poison Information Center (IAPIC)* between January 1986 and August 1988 (Lovell, 1990), although this may reflect its widespread use as much as its toxic potential. Symptoms of neurotoxicity in dogs and cats manifest as muscle tremors, ataxia, and alteration of the patient behavior within 24 hours after a daily dose of 100 mg/kg. No toxic effects have been reported in foals and horses after they were given six or seven times the therapeutic dose. However, four times the therapeutic dose may provoke digestive discomforts such us transitory diarrhea and excessive rumen gas accumulation in cattle. The therapeutic index of piperazine has been established as 3 (cats) to 6 (horses) (EMEA, 2001). Overall, piperazine remains useful as a result of its low cost, safety, and high efficacy against ascarids in different animal species.

Diethylcarbamazine Citrate

Diethylcarbamazine (MW 391.4) (Figure 39.12) is a derivative of piperazine, which is highly soluble in water, alcohol and chloroform, but insoluble in organic solvents (Pharmaceutical Society of Great Britain, 1979); it is stable in the environment. In veterinary medicine, diethylcarbamazine is formulated as tablets or chewables that are sold as a preventive for heartworm disease (*Dirofilaria immitis*) in dogs. Diethylcarbamazine should be administered daily throughout the mosquito vector season and continued for 2 months following. It is also used in combined commercial formulations also containing oxibendazole, ivermectin, or milbemycin oxime. Diethylcarbamazine has also been previously used for the treatment of lungworm infection in ruminants.

Diethylcarbamazine appears to have different mode of action compared with other antiparasitic molecules, since its microfilarial activity is absent in vitro but extensive in vivo. It has been shown that diethylcarbamazine inhibits cyclic peroxide generation from arachidonic acid (AA) breakdown, specifically through its effects on the enzymes leukotriene A4 synthetase (LTA4), prostacyclin 2 synthetase (PGI2), and prostaglandin synthetase

(PGE2). However, diethylcarbamazine has no apparent inhibitory effect on thromboxane synthetase and, therefore, cannot be classified as an inhibitor of cyclooxygenase (Martin, 1997). The microfilaria produces PGI2 and PGE2 within endothelial cells of blood vessels of infested patients. Since diethylcarbamazine can alter AA metabolism in both parasite and host, it is possible that vasoconstriction combined with amplified endothelial adhesion may be responsible for the immobilization of the microfilaria with a complementary cytotoxic action provided by host platelets and granulocytes (Martin, 1997).

After oral administration, diethylcarbamazine is readily absorbed by the GI tract, achieving maximum plasma concentration (C_{max}) at 3 hours postadministration, with a subsequent plasma detection period of approximately 48 hours. The drug is widely distributed in tissues and is metabolized in the liver by N-dealkylation and N-oxidation into four metabolites. Diethylcarbamazine is excreted in the urine either unchanged or as the N-oxide metabolite. Urinary excretion and, consequently, plasma half-life are dependent on urinary pH (Martindale, 1993). Studies with activated charcoal demonstrated that the latter significantly decreased the absorption and elimination of diethylcarbamazine by adsorption in the GI tract (Orisakwe et al., 2000). Diethylcarbamazine was shown to have generally low toxicity at the therapeutic dose of 6.6 mg/kg given as oral tablets, or 2.5 mg/kg orally given in powder form once a month, although gastric irritation was observed as a side effect in a few dogs. Diethylcarbamazine is contraindicated in dogs parasitized with adult filaria since a hypovolemic shock type reaction may occur in a small percentage (0.3–5%) of such dogs after diethylcarbamazine administration, which may lead to death within a few hours (Courtney and Roberson, 1995).

Heartworm Adulticides: Organic Arsenicals

Thiacetarsamide Sodium

This compound is a disodium salt of the *S,S*-diester of *p*-carbamoydithiobenearsonous acid with mercaptoacetic acid. It is recommended to evaluate the liver and kidney function before dosing since this arsenical compound has shown to be toxic. The plasma pharmacokinetics of thiacetarsamide has been reported in dogs after intravenous injection (2.2 mg/kg), displaying a two-compartment open model curve. A wide range in kinetic values were obtained for elimination half-life (20.5 to 83.5 minutes) and clearance rate (80.0 to 350 ml/kg/min). The mode of action is still unclear, but is presumably due to the action of the arsenic on cell glycolisis. Treatments of heartworm (*Dirofilaria immitis)* is its main therapeutic use, although a potential use in chronic fatigue syndrome associated with *Staphylococcus* spp. bacteremia has been described (Tarello, 2001). The compound does not have efficacy against circulating microfilariae. Adult worms die within a week after treatment. A dose of 3.7 mg/kg daily for 3 days produced serious side-effects in a third of the dogs tested. Side-effects at the standard dose increase with the clinical severity of the heartworm disease. Toxicity is revealed by vomiting, icterus, and orange colored urine. Dimercaprol (8.8 mg/kg/day in four divided doses) is the recommended antidote when toxic signs are manifested. Cats have demonstrated sensitivity to thiacetarsamide and its use is not recommended in this species.

Melarsomine

Melarsomine is a trivalent arsenical of the melanonyl thioarsenite family with activity against adult and 4-month-old heartworms in dogs. Melarsomine is available as a powder for mixing with sterile water before use. The FDA approved this molecule for use in dogs under hospital setting. Its mode of action is unknown. It is rapidly absorbed after intramuscular injection (2.5 mg/kg) in dogs, achieving the C_{max} at only 8 minutes postinjection. It has also been reported that melarsomine and its metabolites are free in plasma, unlike thiacetarsamide which binds to red blood cells. Arsenic plasma levels are higher and measured for a longer time after treatment with melarsomine compared to those achieved after thiacetarsamide administration.

Melarsomine is active against immature (>4 month old) and adult heartworms in dogs. Heartworm disease is preventable using the daily or monthly heartworm prevention medications. It is preferred over the use of thiacetarsamide since it can be used more safely in dogs with severe heartworm disease. The side effects provoked after injection were described as pain, swelling, and tenderness at the injection site. Coughing, gagging, depression, lethargy, lack of appetite, fever, lung congestion, and vomiting may also appear as side effects. Melarsomine has a low margin of safety and it is recommended to have an accurate weight before treating. Dimercaprol can be used as a possible antidote for reversing the toxicity within 3 hours.

Macrocyclic Lactones

Ivermectin, moxidectin, and milbemycin oxime are macrocyclic lactone compounds used to kill the tissue larval stages of *Dirofilaria immitis* in dogs and cats. The pharmacology of these exceptionally potent and highly safe compounds is fully discussed in Chapter 41.

Resistance to Anthelmintic Compounds: Molecular Basis

Perhaps it is the simplicity of treating infections with very effective drugs on a routine basis and the proven cost-effective gains in short-term productivity that has led to the predominance of chemotherapy (Zajac et al., 2000). Consequently, resistance to anthelmintic drugs has become a major problem in veterinary medicine, and threatens both livestock income and animal welfare.

Resistance is present when there is a greater frequency of individuals within a population able to tolerate doses of a compound than in a normal susceptible population of the same species. The most important feature of anthelmintic resistance is that it is inherited. Selection contributes to production of resistant populations. For each chemical class of anthelmintics, resistance to one member usually confers resistance to the other members. Genetic modifications conferring resistance are translated into different biochemical modifications that determine a reduced drug effect in the resistant cell. These molecular changes represent the pharmacological basis of the resistance phenomena. Parasites have a number of strategies to become resistant, including: (i) molecular changes affecting the capacity of the drug to accumulate at intracellular site of action (reduced uptake, enhanced active efflux, and metabolism); (ii) modified activity of parasite enzymatic systems; (iii) changes on the number, structure, and/or affinity of cellular drug receptors; and (iv) amplification of target genes to overcome the effect of the anthelmintic drug (Table 39.1).

The mechanism of action determines the time of appearance of the antiparasitic effect and the potential risk for the development of resistance to a given drug chemical class. The appearance of resistance to all the major families of broad-spectrum anthelmintics, namely benzimidazoles, imidazothiazoles (levamisole)/

Table 39.1 Summary of the main anthelmintic resistance mechanisms described in helminth parasites for the most important chemical families: macrocyclic lactones (ivermectin, moxidectin, etc.), benzimidazoles (albendazole, fenbendazole, etc.), and imidazothiazoles (levamisole)

	Macrocyclic lactones	Benzimidazoles	Imidazothiazoles
Pharmacodynamic based mechanisms	Mutations in GluCl receptor genes[a] Mutations in GABA receptor genes[b] Decreased GluCl receptor gene expression[c]	Mutations in isotype-1 β-tubulin genes[f]	Mutations in nACh receptor subunits genes[i] Different combinations of receptor subunits[j] Reduced/increased expression of nACh receptor subunit genes[k] Abbreviated isoforms of nACh receptor subunits genes[l]
Pharmacokinetic based mechanisms	P-GP overexpression[d]	P-GP overexpression[g]	P-GP overexpression[m]
	Reduced drug uptake[e]	Detoxification[h] (enhanced oxidation and drug efflux)	

GluCl, glutamate-gated chloride ion channel receptor; GABA, γ-aminobutyric acid receptor; nACh, nicotinic acetylcholine receptor; P-GP, P-glycoprotein.

[a] Mutations in *GluClα3* subunit gene in resistant *Cooperia oncophora* (Njue et al., 2004).

[b] Mutations in GABA receptor genes on a *Haemonchus contortus* isolate (Feng et al., 2002).

[c] Reduced expression levels of the GluCl receptor genes in *H. contortus* (Williamson et al., 2011), *C. oncophora,* and *Ostertagia ostertagi* (El-Abdellati et al., 2011). However, receptor gene changes appear not to be primarily involved in most cases of field resistance to macrocyclic lactones (Kotze et al., 2014).

[d] Increased drug efflux mediated by overexpression of P-GP (Williamson et al., 2011; Dicker et al., 2011; Janssen et al., 2013; De Graef et al., 2013).

[e] Changes to the anatomy and/or function of amphid sensory endings associated to reduced drug intake (Dent et al., 2000).

[f] Single nucleotide polymorphisms (SNPs) at position 200 (Kwa et al., 1994), 167 (Silvestre and Cabaret, 2002), and 198 (Ghisi et al., 2007) in the isotype-1 β-tubulin gene; SNPs at position 200 appears to be the most important regarding the BZD-resistance phenotype.

[g] Increased drug efflux mediated by overexpression of P-GP in *H. contortus* (Blackhall et al., 2008) and *Fasciola hepatica* (Alvarez et al., 2005).

[h] Increased triclabendazole detoxification in triclabendazole-resistant *F. hepatica* (Robinson et al., 2004; Alvarez et al., 2005).

[i] Mutations in nACh receptor subunit gene observed in *Caenorhabditis elegans* (Fleming et al., 1997).

[j] Different combinations of receptor subunits give raise to receptors with different pharmacological properties (Kotze et al., 2014).

[k] Reduced/increased expression of several nACh receptor subunits genes (Kopp et al., 2009; Sarai et al., 2013; Sarai et al., 2014; Romine et al., 2014).

[l] Abbreviated isoform of a nACh receptor subunits (unc-63) in *H. contortus*, *Teladorsagia circumcincta,* and *Trichostrongylus colubriformis* (Neveu et al., 2010).

[m] Increased expression of several P-GP genes in levamisole-resistant *H. contortus* larvae (Sarai et al., 2014).

tetrahydropirimidines (pyrantel, morantel), and macrocyclic lactones (avermectins, milbemicyns), has strongly motivated the development of pharmacodynamic studies in order to understand the mechanisms of drug action and resistance. Table 39.1 summarizes the main resistance mechanisms described for the most important anthelmintic chemical families.

As described in this chapter, the nematodicidal action of the benzimidazole (BZD) anthelmintics is based on their selective binding to β-tubulin, which produces subsequent disruption of the tubulin–microtubule dynamic equilibrium. BZD resistance in most trichostrongylid nematode species has been associated with the loss of high-affinity binding receptors and an alteration of the β-tubulin isoform pattern (Lubega and Prichard, 1991) based on a well-conserved mutation (single nucleotide polymorphism, SNP) at amino acid 200 (phenylalanine to tyrosine) in the parasite's isotype-1 β-tubulin gene (Kwa et al., 1994; Prichard, 2001). Others BZD-resistance-associated SNPs have been reported at codons 167 (Silvestre and Cabaret, 2002) and 198 (Ghisi et al., 2007). Additionally, modulation of the activity of transmembrane efflux pumps such as P-glycoprotein (P-GP) could also contribute to BZD resistance in trichostrongyles (Kerboeuf et al., 2003).

Levamisole acts selectively as an agonist of nicotinic acetylcholine (nACh) receptors, a transmembrane ion (Na^+ and Ca^{++}) channel, composed by five subunits. More than 30 different nACh receptor subunits has been described in parasites (Laing et al., 2013), allowing the formation of different pentameric structures. Different combinations of receptor subunits give rise to receptors that have different pharmacological properties. For example, some receptors could be sensitive to levamisole but less sensitive to pirantel and viceversa. A reduced expression of several nAChR genes has been associated with a decreased sensitivity to pyrantel in *Ancylostoma caninum* (Kopp et al., 2009). Resistance to cholinergic agonists in parasitic nematodes is polygenic and involves changes in expression of the nAChR subunits, truncated receptor subunits, and mutations in receptor subunits, as well as a possible contribution from P-GPs (Kotze et al., 2014). As an example, Sarai et al. (2014) examined subpopulations within a heterogeneous isolate of *Haemonchus contortus* and found that expression of several P-GP genes was increased in larvae showing a low level of resistance to levamisole. Furthermore, in the most resistant larvae within the population, expression of some receptor subunits genes was decreased (Sarai et al., 2014), which may indicate multiple mechanism of resistance within the same population.

It is important to note that nematodes resistant to levamisole are also resistant to the tetrahydropirimidine compounds (morantel, pyrantel). Although chemically different, morantel and pyrantel share the same mode of action with levamisole (see Figure 39.11) and therefore side resistance among them has been demonstrated and described elsewhere.

Resistance to the avermectin and milbemycin macrocyclic lactones had been related to selection of genes related to the expression of some subunits of the glutamate-gated chloride channels (GluCl) and to the drug transport P-GP (see Chapter 41 for detailed information). Additionally, genes related to a GABA-gated chloride channel subunit and β-tubulin seem to be also associated with resistance to the macrocyclic lactone compounds (Eng and Prichard, 2005). In spite of the involvement of different genes on the resistance to these molecules, not all of them may contribute to the same extent in different nematode-resistant strains. For instance, there is no evidence that polymorphism of GluCl or GABA receptors can explain the observed resistance to macrocyclic lactones compounds in most field isolates of different gastrointestinal parasites (Kotze et al., 2014).

P-GP is a member of the ATP-binding cassette (ABC) group of transporters that function as ATP-dependent efflux mechanism, enabling drugs to be expelled from cells. P-GP overexpression is a common pattern on the multidrug resistant (MDR) phenotype in different cancer cells. Overexpression of P-GP has also been implicated in the resistance of nematodes to ivermectin and moxidectin (Pouliot et al., 1997), closantel, and BZD, although the exact nature of its role has yet to be established. P-GP genes have been identified in *Caenorhabditis elegans* (at least 14 genes) (Lincke et al., 1992), in *Haemonchus contortus* (seven genes) (Sangster et al., 1999) and *Onchocerca volvulus* (two genes) (Kwa et al., 1998). In trematodes, genes encoding ABC proteins have been identified in *Schistosoma mansoni* (Bosch et al., 1994) and *Fasciola hepatica* (Reed et al., 1998). The macrocyclic lactones interact with P-GP and ivermectin has been used as an MDR-reversing agent. It has been shown that *Haemonchus contortus* resistant to ivermectin possess an increased level of P-GP expression (Xu et al., 1998) and coadministration with verapamil (an MDR-reversing agent) increase the efficacy of ivermectin and moxidectin against resistant *Haemonchus contortus* (Molento and Prichard, 1999). Phenotypic and genotypic evidences of macrocyclic lactones resistance associated with P-GP single nucleotide polymorphic sites in *Dirofilaria immitis* have been described (Bourguinat et al., 2011). The resistant filarial nematode was isolated from a dog that remained microfilariemic despite successful adulticidal treatments and repeated treatment with high doses of macrocyclic lactones. The genetic polymorphism (GG–GG), previously found to be associated with insensitivity to macrocyclic lactones in vitro, was present at a frequency of 45.3% in microfilariae that survived repeated treatments with high doses of ivermectin. The interested reader should consult Chapter 50 for a general background on pharmacogenomics applied to a wide variety of drugs.

Roundworms have sensory neurons (amphidial neurons) in their cephalic end. They are located in a pair of channels (the amphids) on either side of the pharynx (Freeman et al., 2003). The amphid dye filling defective (Dyf) gene, osm-1, and other Dyf genes, may act additively to regulate ivermectin uptake in *Caenorhabditis elegans* (Dent et al., 2000). Furthermore, Dyf mutations confer low level of resistance to ivermectin. Interestingly, the amphids structure is altered in ivermectin-resistant *Haemonchus contortus* (Dent et al., 2000), suggesting that a decreased ivermectin entry into the nematode may be other mechanism involved in drug resistance.

Anthelmintic resistance is a worldwide problem, which mainly involves nematode parasites from sheep, goats, and horses. However, there are already cattle nematodes resistant to multiple anthelmintic classes in New Zealand and South America (Fiel et al., 2000; Mejía et al., 2003; Loveridge et al., 2003; Gasbarre, 2014) and this will probably become more widespread. The rate at which anthelmintic resistance develops and spreads within a population is determined by the amount of parasites that survives the anthelmintic treatment, and contribute with their resistance genes to the next generations. This contribution is mainly influenced by parasite genetic factors, fecundity of adult parasites (generation time, offspring per generation, breeding patterns), parasites in refugia, drug features (frequency and timing of treatments, drug persistence in the host, drug dose and efficacy), rate of consumed larvae, grazing management strategy and climatic conditions. Refugia are subpopulations of parasites that are not selected by drug treatment. Organisms in the environment (larvae on pastures), which are not exposed to drugs, are in refugia. They are important because the higher the proportion of the population in refugia, the slower the selection for resistance (Sangster, 2001).

Due to the great effort involved in the development of new anthelmintic molecules, it is crucial to optimize the use of the existing ones. Increasing drug bioavailability is a pharmacological tool that cooperates with the optimization of the treatment, thereby delaying the development of anthelmintic resistance. Any pharmacological tool that allows the enhancement of a drug's systemic bioavailability will facilitate achieving higher drug concentrations to reach the sites of parasite location, for sufficient period of time, to achieve the antiparasitic effect, especially for those carrying the resistant genes. This is applicable to only those mechanisms of resistance that depend on drug concentration. A number of strategies for optimizing the use of available anthelmintics and to delay the development of resistance are under investigation, and include: (i) use of different pharmacotechnically based approaches to enhance drug absorption; (ii) enhancement of drug systemic availability by feed management (fasting before/after treatment); (iii) pharmacological modification to diminish drug efflux from resistant parasites; and (iv) modification of drug metabolism/elimination.

The use of a drug class that has been shown to be effective, or a combination of drugs with different modes of action, combined with rotation among chemical groups, and the use of as few drug treatments as possible, are the most practical and viable recommendations at present to delay the development of resistance. A major challenge is to define strategies to preserve susceptibility. Monitoring parasite populations and treating the animals when numbers reach a threshold are practical options suggested to slow down the spread of resistance (Sangster, 2001). Early detection of resistance and provision of information to farmers and their advisors are essential features of parasite control strategies aimed at preserving drug efficacy. In conclusion, integrated pharmacoparasitological research is required to minimize the impact of resistance and to obtain a complete understanding of the basic biology of the parasites and the strategies they develop for becoming resistant. Such an approach may help to identify solutions for delaying the development of resistance to the current available anthelmintics.

Nematodicidal Drug Combinations

Drug combination has been used as a useful tool to increase anthelmintic spectrum (i.e., ivermectin + clorsulon combination, which expand the spectrum of ivermectin to adult liver flukes). Furthermore, since multidrug resistance is becoming a widespread problem in farm animals, the use of drug combinations may be a practical approach to delay the development of resistance. Mixtures of drugs from different chemical families have been proposed as a valid strategy to delay the development of anthelmintic resistance (Anderson et al., 1988). Currently, a large number of combined anthelmintic drenches are available in the veterinary pharmaceutical market of important sheep-producing countries such as Australia, New Zealand, and Uruguay. Some broad-spectrum antiparasitic combinations containing albendazole, ivermectin, and levamisole (Triton®, Merial) or oxfendazole, abamectin, and levamisole (Matrix®, Ancare) have been initially introduced into the Australian and/or New Zealand markets. A multicombination drench for sheep, which combines albendazole, levamisole, closantel, and abamectin (Q-Drench®, Jurox) is approved for use in sheep in Australia.

The rationale behind using drug combinations is based on the fact that individual worms may have a lower degree of resistance to a multiple component formulation (each chemical with different mode of action/resistance) compared to that observed when a single anthelmintic molecule is used. The use of these formulations is based in a lower resistance in individual worms to a preparation with multiple components (each one with different mechanism of action) compared to the treatment with a formulation with a single active component. In an ideal situation, if an anthelmintic treatment reaches 100% of efficacy, selection of anthelmintic resistance will never occur. To achieve the highest efficacy in treated animals while the few surviving parasites are diluted into a susceptible untreated nematode population is a key principle for slowing the emergence of anthelmintic resistance in a real field situation (Dobson et al., 2001). Consequently, in farms where multiple-resistant nematode populations are present, the use of drug combinations may be an alternative to improve chemical control. Anthelmintic combinations can be used for delaying anthelmintic resistance, for specific targeting of dose-limiting species, and for managing existing resistance (Geary et al., 2012; Bartram et al., 2012). It has been reported that after the use of either a triple (levamisole + albendazole + ivermectin) combined treatment or ivermectin alone, a similar nematode control was observed (Suarez et al., 2014). Thus, the use of drug combinations to manage anthelmintic resistance may not always be an alternative to improve chemical control. Clearly, under a number of field situations, the use of anthelmintic combinations in sheep production (where anthelmintic resistance is common) may have limited sustainability. However, in cattle production systems where individual molecules still maintain their highest efficacy, the combined use of anthelmintics may be an important tool to delay resistance.

The occurrence of potential drug interactions between drug components highlights the need for deeper pharmacological-based research to identify the advantages/disadvantages of the use of combined drug preparations for anthelmintic control in livestock. A potential drug interaction refers to the possibility that one drug may alter the intensity of the pharmacological effects of another drug given concurrently. The modified effect may result from a change in the concentration of either one or both drugs in the organism (pharmacokinetic interaction) or from a change in the relationship between drug concentration and response of the organism to the drug (pharmacodynamic interaction) (Figure 39.13). There are four possible types of pharmacodynamic interactions resulting on either additive, potentiating (synergistic or supraadditive), antagonism (infraadditive), or indifferent effects. An additive effect arises when the combined effects of two drugs equal the sum of their independent activities measured separately. On the other hand, a potentiating effect is achieved when the combined effects of the drugs are significantly greater than the sum of the independent effects. A potentiating effect obtained after the coadministration of two drugs with different mode of action would be an ideal strategy to combat resistant parasites, particularly in cases of simultaneous multiple resistance, characterized by the presence of one or more worm genera resistant to all the molecules included in the combination. Since levamisole, albendazole, and ivermectin differ in their intrinsic anthelmintic mode of action, their coadministration may potentially induce a synergistic effect. Evidence of synergist action between the BZD compound fenbendazole and levamisole has

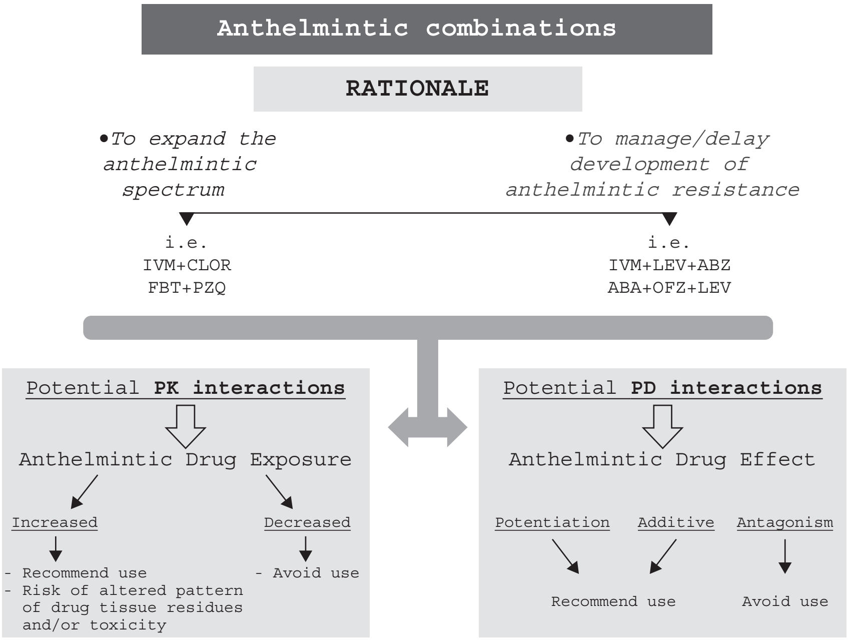

Anthelmintic combinations
RATIONALE
•To expand the anthelmintic spectrum
•To manage/delay development of anthelmintic resistance
i.e.
IVM+CLOR
FBT+PZQ
i.e.
IVM+LEV+ABZ
ABA+OFZ+LEV
Potential PK interactions
Anthelmintic Drug Exposure
Increased
Decreased
- Recommend use
- Risk of altered pattern of drug tissue residues and/or toxicity
- Avoid use
Potential PD interactions
Anthelmintic Drug Effect
Potentiation
Additive
Antagonism
Recommend use
Avoid use

Figure 39.13 Combined use of anthelmintic drugs. Rationale and potential pharmacokinetic (PK) and pharmacodynamic (PD) drug to drug interactions. IVM, ivermectin, CLOR, clorsulon, FBT, febantel, PZQ, praziquantel, LEV, levamisole, ABZ, albendazole, ABA, abamectin, OFZ, oxfendazole.

been described (Miller and Craig, 1996). Furthermore, a synergistic interaction between the novel anthelmintic derquantel and abamectin appears to occur under in vitro laboratory conditions (Puttachary et al., 2013). However, most cases of pharmacodynamic interactions between nematodicidal drugs appear to be limited to an additive effect. When multiple resistance refers to the presence of different worm genera, each one resistant to one different anthelmintic chemical family, a greater anthelmintic efficacy could be obtained by using the combination compared to that achieved by the use of each component alone, since worms surviving one molecule could be eliminated by the other. In this situation, an additive effect is apparently involved in the increased efficacy observed for the combination of albendazole sulfoxide and levamisole against BZD-resistant nematodes (Anderson et al., 1991). However, field work undertaken in Uruguay (Suarez et al., 2014) showed an equivalent efficacy against multiple-resistant *Haemonchus contortus* following the use of either a triple combined treatment (levamisole + albendazole + ivermectin) or ivermectin alone, indicating no advantageous effect of the triple combined preparation. Drug combination use requires previous diagnosis of the resistance status and it should be avoided once resistant to all the combined molecules is present, since it may have limited success reversing the resistant status.

A pharmacokinetic interaction occurs when a drug compound modifies the absorption, distribution, biotransformation, and/or elimination of another drug. As a consequence, the drug concentration at the biophase (the site of action) may be either increased or decreased. Since the higher the concentration achieved at the tissue where the parasite is located, the greater the amount of drug reaching the target parasite receptor pharmacokinetic interactions may determine modified anthelmintic efficacy. As a common feature, a drug to drug pharmacokinetic interaction can be observed after the coadministration of two or more anthelmintics, as was described in the combined administration of albendazole plus ivermectin (Alvarez et al., 2008), rafoxanide plus ivermectin (El-Banna et al., 2008), and levamisole plus albendazole plus ivermectin (Suarez et al., 2014). However, there are some reports on the absence of pharmacokinetic interactions between anthelmintics used as combinations. For instance, no differences on the plasma pharmacokinetic parameters were observed for ivermectin and closantel, administered by SC injection each alone or as a combined formulation to cattle (Cromie et al., 2006). The assessment of either the positive or negative (adverse) impact of pharmacokinetic interactions occurring between combined anthelmintic molecules needs to be elucidated before these drug mixtures are introduced into the market.

Novel Anthelmintic Drugs

The development of resistance to the existing chemical classes of broad-spectrum anthelmintics is a primary challenge for discovering new drugs to assure the long-term viability of the animal health industry (Hennessy, 1997). This situation highlights the need for developing new antiparasitic compounds with unique modes of action.

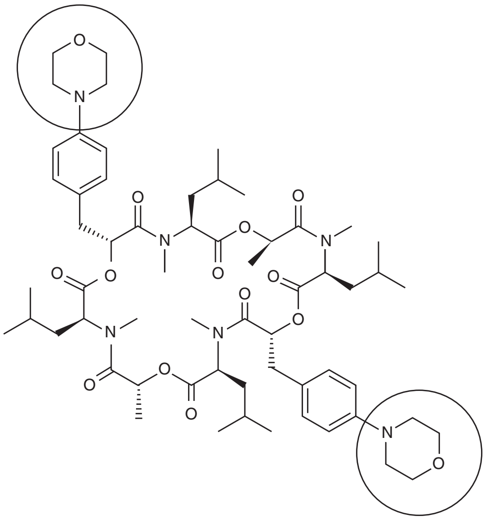
Nitazoxanide is a salycilanilide derivative of nitrothiazole. Nitazoxanide is effective against a broad spectrum of parasites and viruses infecting animals and humans, including nematodes, cestodes, trematodes, coccidian, ciliate, and flagellate protozoa. Its antiparasitic activity in domestic animals was reported a long time ago (Euzeby et al., 1980) but the efficacy pattern is too low to be attractive to be marketed in veterinary medicine. The mode of action for killing worms, protozoa, and some viruses is unknown but its comprehension may offer an opportunity for the discovery of analogs with improved potency and efficacy (Geary et al., 1999a).

The cost of developing a new class of broad-spectrum anthelmintics for use in food-producing animals is rapidly becoming prohibitive (Hennessy, 1997). However, in the last two decades numerous pharmacological studies have been carried out to generate new promising molecules with potent and unique pharmacological activity. The diketopiperazines (*marcfortine* and *paraherquamide A*), cyclic octadepsipetides (*PF1022, emodepside*), neuropeptides *(FaRP),*and the aminoacetonitrile derivatives (AADs) are among the most important novel antiparasitics. Although there is some information on the specific site of action for most of these novel molecules, the exact mechanism of action for some of them remains to be fully understood.

Paraherquamide has excellent activity against almost all of the major parasites of sheep (Shoop et al., 1990). Furthermore, oxindole alkaloids in the paraherquamide/marcfortine family exhibit broad-spectrum anthelmintic activity, including drug-resistant nematode strains (Zinser et al., 2002). Paraherquamide and related anthelmintics act as antagonists of neuronal nicotinic cholinergic receptors in both nematodes (flaccid paralysis in vitro) and mammals, and this mechanism appears to underlie both their efficacy and toxicity. 2-Desoxoparaherquamide, known as derquantel is a promising PHQ semisynthetic derivative active against both BZD and ivermectin-resistant *Haemonchus contortus* that shows markedly lower affinity for mammalian receptors than paraherquamide itself (Geary and Thompson, 2003). Derquantel is well absorbed and extensively distributed into tissues after oral administration, showing excellent anthelmintic activity against *H. contortus* (adults stages), *T. colubriformis,* and *Nematodirus* spp. (adults and L4 stages) (Little et al., 2011). In an attempt to optimize its anthelmintic activity and to decrease the selection pressure on resistant nematode strains, derquantel has been launched for use in combination with abamectin (see Chapter 41, Macrocyclic Lactones) in New Zealand, Australia, Uruguay, etc.

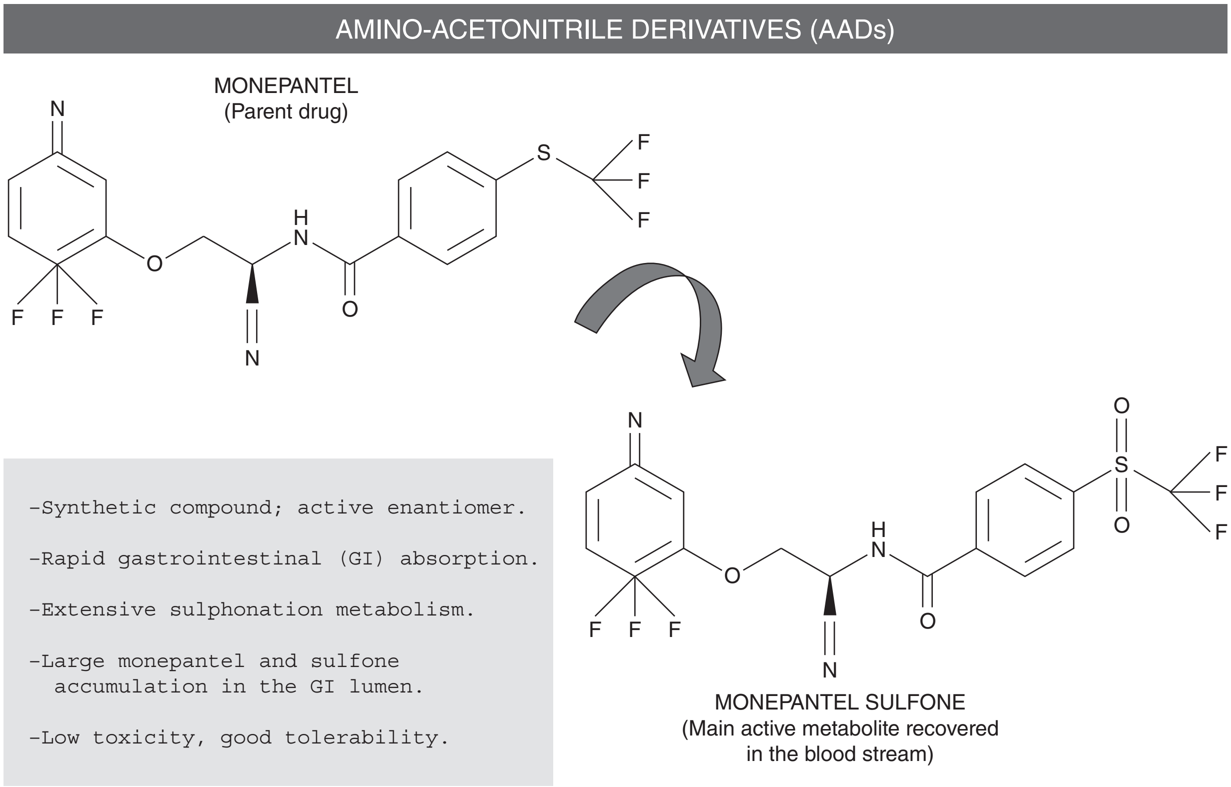
PF1022A (Sasaki et al., 1992), the mother compound of the class of the N-methylated 24-membered cyclooctadepsipeptides obtained as a secondary metabolite from the fungus *Mycelia sterilia*, demonstrates exceptional activity against a variety of nematodes in vitro and in vivo (Conder et al., 1995; von Samson-Himmelstjerna et al., 2000). It has been reported that PF1022A paralyses the worms by acting as GABA agonist (Chen et al., 1996) and cholinergic antagonist simultaneously (Harder et al., 2005). However, difficulties have been reported regarding its delivery to ruminant animals. Emodepside is a semisynthetic derivative of PF1022A which contains a morpholine molecule attached in the *para* position at each D-phenyllactic acid (Figure 39.14). This novel anthelmintic molecule is efficacious against a variety of GI nematodes. Emodepside acts presynaptically on a latrophilin-like receptor (depsiphilin, a putative G protein coupled receptor), located in the pharynx and body wall muscle of nematodes, which induces activation of a signal transduction cascade via Gq-alfa-protein and phospolipase-C-beta causing an increase in intracellular calcium and diacylglycerol (DAG) levels. DAG activates proteins that play a role in presynaptic vesicles function, which leads to the release of an inhibitory neuropeptide that acts postsynaptically, inducing flaccid paralysis in both pharyngeal and somatic body wall muscles of the nematode (Harder et al., 2005). Emodepside has a novel mode of anthelmintic action and is fully effective against benzimidazole, levamisole, or ivermectin-resistant nematodes of sheep and cattle (Harder et al., 2005). Emodepside is efficacious in cats against nematodes, either following its administration alone or in combination with praziquantel, which has no nematodicidal activity. At 3.0 mg/kg emodepside (spot-on formulation) has excellent activity against *Toxacara cati* (mature and immature adults), *Toxascaris leonina,* and *Ancylostoma tubaeforme* (Altreuther et al., 2005).

FMRFamide-related peptides (FaRPs) are neuropeptide with motor-modulatory activities in both arthropods and helminths. Progress in the characterization of invertebrate neuropeptide receptors has demonstrated that neuropeptide signaling may be a sound approach to identify novel endectocide molecules (Mousley et al., 2004a). FaRPs play a key role in motor coordination, which has been demonstrated in arthropod, flatworm, and nematode nervous systems (Geary et al., 1999b).



-New anthelmintic class: N-methylated 24-membered cyclo-octadepsides
-Semi-synthetic derivative of PF1022A (circled areas show the morpholine moiety in paraposition of the parent compound)
-Novel mode of action
-Broad nematocidal spectrum (larval and adult stages)
-Active against nematodes resistant to benzimidazoles, levamisole and ivermectin

Figure 39.14 Chemical structure and main pharmacological properties of emodepside, a novel anthelmintic molecule. Emodepside in combination with the cestodicide praziquantel is commercially available for use in cats (spot-on) and dogs (tablets).


AMINO-ACETONITRILE DERIVATIVES (AADs)
MONEPANTEL
(Parent drug)
MONEPANTEL SULFONE
(Main active metabolite recovered
in the blood stream)

-Synthetic compound; active enantiomer.

-Rapid gastrointestinal (GI) absorption.

-Extensive sulphonation metabolism.

-Large monepantel and sulfone accumulation in the GI lumen.

-Low toxicity, good tolerability.

Figure 39.15 Chemical structures and main pharmacological features of the novel nematodicidal compound monepantel and its sulfone metabolite. Source: Data from Kaminsky et al., 2008; Karadzovska et al., 2009; Lifschitz et al., 2014.

FaRPs are the most potent peptides, supporting their selection as the strongest candidates for chemotherapy intervention (Mousley et al., 2004b). In association with their marked structural diversity, FaRPs within each phylum display either myoexcitatory (flatworms) or a variety of myomodulatory effects (arthropods and nematodes) that are dependent upon peptide structure (Mousley et al., 2004a). FaRPs receptors (G-protein coupled receptors) may present a suitable target for new anthelmintics (Geary et al., 1999b). FaRP receptors (or the tissues that express them) in arthropods, flatworms, and nematodes are promiscuous with respect to the interactive peptide ligands. In fact, an individual peptide from a nematode or arthropod source can activate receptors involved in motor function within the three phyla. The interphyla activity data support the concept of a single drug being able to interfere with FaRP signaling across the phyla that encompass the metazoan parasites of importance (Mousley et al., 2004a).

The molecules described here represent encouraging classes of novel antiparasitics due to their particular mechanisms of action. They may constitute a solution to control parasites resistant to the available drugs. However, a series of difficulties (toxicity, formulation, etc.) must be overcome before the most of them can reach commercial development. For instance, emodepside has been introduced in combination with praziquantel as a broad-spectrum anthelmintic for cats (spot-on) and dogs (oral) but its development for use in other animal species requires further work.

The discovery of the aminoacetonitrile derivatives (AADs) as a new chemical class of synthetic anthelmintics and the development of monepantel (Figure 39.15) are relevant steps forward in research to increase the available therapeutic options to control gastrointestinal nematodes (Kaminsky et al., 2008). A significant number of AAD compounds (about 700) were evaluated (Kaminsky et al., 2008) and finally monepantel was introduced into the veterinary pharmaceutical market. Monepantel was registered in Australia, New Zealand, and in some South American countries. Monepantel is active against larval and adult stages of gastrointestinal nematodes of sheep and cattle. It is highly effective against nematodes resistant to all the other available anthelmintic families. Monepantel effectiveness is based on a novel mechanism of action involving a unique, nematode-specific clade of the nicotinic acetylcholine receptors, which allows existing resistance to the currently available anthelmintics to be overcome. Genetic studies, conducted on the free-living nematode *Caenorhabditis elegans* and *Haemonchus contortus*, showed that monepantel acts as an agonist at the nicotinic acetylcholine receptor, producing spastic paralysis and death of the worm (Kaminsky et al., 2008; Rufener et al., 2010). After its intravenous and oral administration to sheep, monepantel is rapidly converted into a monepantel sulfone metabolite (Karadzovska et al., 2009). It has been suggested that monepantel sulfone may have the same in vitro anthelmintic activity as monepantel parent drug (Karadzovska et al., 2009). Thus, the large accumulation of both monepantel parent compound and its sulfone metabolite in different sections of sheep digestive tract (Lifschitz et al., 2014) may be relevant to the efficacy against different gastrointestinal nematodes. The activity against multidrug-resistant isolates based on a novel mode of action, its good tolerability, and low mammalian toxicity are highly favorable elements for the future of monepantel for nematodes control. However, some specific knowledge on its pharmacological behavior, as well as a highly responsible use, will be necessary to secure its maximum lifespan. The emergence of new antiparasitic compounds into the veterinary pharmaceutical market makes it necessary to have a deep understanding of their pharmacological properties in order to avoid their misuse, and thus delay the appearance of resistance

Concluding Remarks

To maximize the efficacy of anthelmintic compounds against parasites that are difficult to control in human and veterinary medicine, whilst preserving an adequate margin of safety, a complete understanding of their pharmacokinetic and metabolic patterns in the host is necessary. The complex connections among route of administration, formulation, drug physicochemical properties, and the resultant kinetic behavior need to be understood to optimize the efficacy of the existing anthelmintics. Additionally, knowledge on the differential drug pharmacological behavior among animal species and identification of different factors affecting drug activity is relevant for achieving optimal parasite control and avoiding selection for drug resistance. The technical information summarized in this chapter illustrates the complexity of the pharmacology of antinematodal compounds in the different animal species. Considering the increasing concern for the development of resistance, the use of pharmacology-based information is critical to design successful strategies for the future of parasite control. Integrated evaluation of the available knowledge on pharmacological features is required to optimize the activity and to achieve rationale use of the existing and novel nematodicidal drugs.

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40

Anticestodal and Antitrematodal Drugs

Carlos E. Lanusse, Luis I. Alvarez, and Guillermo L. Virkel

Anticestodal Drugs

Introduction

Anticestodal drugs used to treat tapeworm infections may act as either taeniafuges or taeniacides. The former compounds facilitate tapeworm expulsion from the host, whilst the later cause the death of the tapeworm in situ. Because the cestode's scolex is capable of regenerating an entire organism, drugs that merely remove the proglottids or the whole tapeworm body but leave the scolex intact are unsatisfactory. Insufficient drug diffusion into the scolex and consecutive two or three proglottids, due to the inflammatory reaction of the intestinal mucosa, may account for such a limited cestocidal activity. The usual interval from destrobilization to patency is approximately 3 weeks, so the recommended interval for evaluating tapeworm therapy is ~3 weeks after initial drug treatment.

Tapeworm infection in farm animals may be a minor problem and usually does not require treatment with a specific cestocidal drug. Some of the broad-spectrum benzimidazole compounds used to control nematode infections (see Chapter 39) are also effective against tapeworms. However, special attention is given to the treatment of *Anoplocephala perfoliata* infections, the commonest tapeworm parasite in horses. Increasing evidence is available suggesting an association between this tapeworm and erosions around the ileocecal valve, intussusceptions, ileal impactions, and other conditions leading to recurrent episodes of intestinal colic (Gasser et al., 2005). It has been shown that oral administration of a pyrantel pamoate paste (see Chapter 39) at 13.2 mg/kg resulted in effective (96%) control of this tapeworm in naturally infected horses (Reinemeyer et al., 2006).

Treatment of tapeworm infections is also necessary in companion animals. It is medically significant that dogs and cats are definitive hosts of tapeworms whose larval stages cause zoonosis. For example, effective treatment of *Echinococcus granulosus* in dogs is extremely important since the larval stage of this tapeworm may cause hydatidosis in the intermediate hosts, primarily sheep and humans. Environmental control of intermediate hosts is also essential to prevent reinfection after treatment. The control of fleas and lice that vector *Dipylidium caninum*, and restricted access of carnivores to mammalian intermediate hosts to preclude *Taenia* infections, are essential (Reinemeyer and Courtney, 2001).

Taeniafuge compounds interfere with the ability of tapeworms to maintain their site of location in the gut. They paralyze tapeworms at least temporarily, but if tapeworms recover prior to expulsion, reattachment to the gut may occur. Thus, treatment with taeniafuges has been routinely accompanied by purgation.

Arecoline, a muscarinic agonist, induces parasite's spastic paralysis and enhances host gut motility, which facilitates tapeworm expulsion. The search for enhanced efficacy motivated the development of synthetic taeniacidal drugs that are now widely used. Many of the older antitapeworm drugs are natural organic compounds (taeniafuges). Readers interested on specific information on those older antitapeworms are referred to a previous edition of this textbook (Roberson and Courtney, 1995).

Bunamidine

Bunamidine (*N*,*N*-dibutyl-4-(hexyloxy)-1-naphthamidine) (Figure 40.1) is a taeniacidal drug that disrupts the tapeworm's tegument and reduces glucose uptake. Consequently, subtegumental tissues are exposed and destroyed by the host digestive enzymes. It is formulated as tablets (hydrochloride salt) or suspensions (hydroxynaftoate salt) for oral administration in companion animals and ruminants, respectively. The hydrochloride salt is widely used for treatment of cestode infections in dogs and cats. Bunamidine hydrochloride exhibits >90% efficacy against *Taenia* spp., *Dipylidium* spp., *Mesocestoides* spp., and *Diphyllobothrium* spp. (oral treatment at 25–50 mg/kg). However, efficacy against *D. caninum* may be erratic. Effectiveness ranging from 85.9 to 98.8% (immature stages) and 100% (mature stages) of *E. granulosus* have been reported (Andersen et al., 1975).

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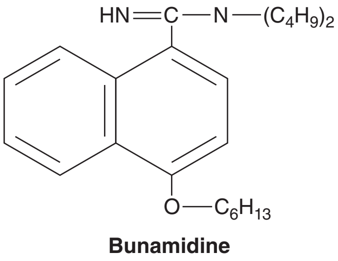


Figure 40.1 Bunamidine chemical structure.

Bunamidine prepared as a hydroxynaftoate salt is effective against *Moniezia spp.* infections in small ruminants.

Dissolution of tablets in fasted animals is improved and, therefore, subsequent contact of the drug with the parasite in the gut is enhanced, resulting in advantageous efficacy. The drug is safe because is not extensively absorbed in the duodenum and low amounts of the absorbed compound are inactivated in the liver. Administration of dissolved tablets enhances drug absorption and, consequently, the risk of systemic adverse effects such as liver damage and ventricular fibrillation. Deaths as consequence of cardiac failure have been sporadically reported within 24 hours posttreatment. Emesis and diarrhea are the most frequent adverse effects following bunamidine administration. More efficacious cestodicidal drugs have displaced bunamidine, which is no longer available in the US market.

Niclosamide

This salycilanilide compound(5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-hydroxybenzamide (Figure 40.2) was widely used for treatment of cestode infections in dogs and cats from the 1960s to the 1980s. Niclosamide is highly effective against most of the tapeworm species in companion animals. However, it has poor efficacy against *Dipylidium* spp. and *E. granulosus*. This drug is also effective for the treatment of common tapeworms in ruminants such as *Moniezia* spp. and *Thysanosoma* spp. In horses it may be used for the treatment of *Anoplocephala* spp. Niclosamide's taenicidal activity involves interference with glucose absorption, inhibition of oxidative phosphorylation, and stimulation of adenosine triphosphatase activity at mitochondrial level. All these mechanisms lead to the death of the parasite and its subsequent digestion within the gut (Al-Hadiya, 2005). It may be administered as either suspension by oral or intraruminal routes in ruminants or as tablets for oral treatment in companion animals. Niclosamide administration in fasted animals increases its efficacy. Its oral administration, at doses of 100–157 mg/kg, produced only low plasma concentrations, which correlates with low gastrointestinal (GI) absorption and the well-known safety of the drug. The absorbed drug is rapidly inactivated in the liver into aminoniclosamide. Similarly to bunamidine, niclosamide has been replaced in small animal practice by modern cestocides. Interested readers are referred to a previous edition of this text (Roberson and Courtney, 1995) for a thorough discussion of the therapeutic properties of niclosamide in animal species of veterinary interest. Interestingly, niclosamide has been identified as a potential anticancer agent due to its inhibitory effects on multiple intracellular signaling pathways. The signaling molecules in those pathways may be over-expressed, constitutively active, or mutated in different cancer cells (Li et al., 2014). Consequently, a number of *in vitro* and *in vivo* studies have reported on the anticancer effectiveness of niclosamide, used either alone or in combination with other antineoplastic agents.

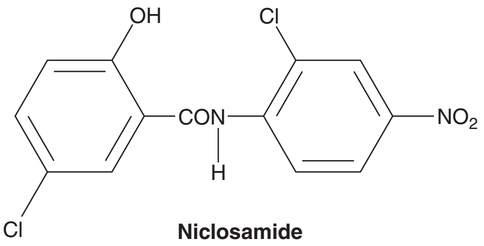


Figure 40.2 Chemical structure of the salicylanilide drug niclosamide.

Praziquantel

Praziquantel is a synthetic isoquinolinepyrazine derivative: 2-(cyclohexykcarbonyl)-1,2,3,6,7,l1b-hexahydro-4*H*-pyrazino[2,1-a] isoquinolin-4-one (Figure 40.3).

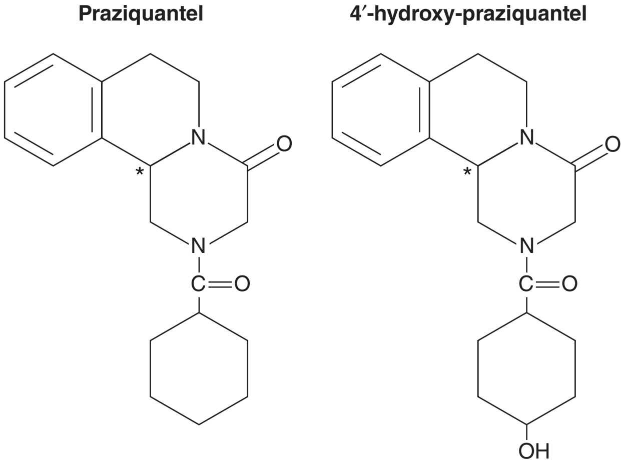


Figure 40.3 Chemical structures of praziquantel and its active metabolite (4′hydroxy-praziquantel). The asterisk (*) indicates the asymmetric (chiral) center in both molecules.

Praziquantel is highly efficacious against a variety of cestode and trematode parasites and it is widely used in both veterinary and human medicine. It has extremely high activity against adult stages of all species of tapeworms in farm and companion animals (Thomas and Gonnert, 1978). It also has good activity against larval forms of cestodes. Administration of praziquantel at 5 mg/kg in cats and dogs is completely effective against all stages of *Taenia hydatigena*, *T. pisiformis*, *T. ovis*, *T. taeniaeformis*, *D. caninum*, *Mesocestoides corti*, *E. multilocularis,* and *E. granulosus* (Thomas and Gonnert, 1978). This dose is generally recommended for elimination of the common cestode species of dogs and cats, except *Spirometra mansonoides* and *Diphyllobothrium erinacea,* which require 25 mg/kg on each of 2 consecutive days. A 10 mg/kg dosage is required to achieve good efficacy against the juvenile forms of these parasites. The drug ensures 100% elimination of *E. granulosus* in dogs and is the unique drug recommended for the treatment of this tapeworm. Oral administration of a praziquantel-containing paste (at 50 mg/kg) was effective for the control of *Dicrocoelium dendriticum* in llamas (Dadak et al., 2013). A combined formulation of febantel and praziquantel is available on the veterinary market for use in the treatment of GI nematodes and cestodes. This formulation must be administered on 3 consecutive days, at doses of 10–15 mg/kg (febantel) and 1–1.5 mg/kg (praziquantel) for adult and young dogs. A triple combination of praziquantel, pyrantel, and oxantel is also marketed as broad-spectrum dewormer for dogs and cats. Other commercial combinations used in companion animals include praziquantel plus oxibendazole, mebendazole, or fenbendazole. Combinations of praziquantel with either ivermectin (paste) or moxidectin (gel formulation) are commonly available as broad-spectrum antiparasitic formulations for use in the treatment of GI nematode and cestode infections in horses (Holm-Martin et al., 2005; Rehbein et al., 2007). A combined spot-on preparation of praziquantel and the novel compound emodepside (see Chapter 39) has been introduced into the market as a broad-spectrum anthelmintic for cats. This anthelmintic formulation has been proven to be effective for the treatment of the feline lungworm *Aelurostrongylus abstrusus* (Traversa et al., 2009).

Praziquantel is also highly effective against cestodes of ruminants. All species of *Moniezia, Stilesia,* and *Avitellina* of sheep and/or goats are eliminated by a single dose of 10–15 mg/kg. Praziquantel has been approved for tapeworm treatment in horses in different countries. It has been successfully used for the treatment of the cestode *A. perfoliata* in this species (Lyons et al., 1998). Combined formulations (gel, paste) containing ivermectin plus praziquantel are marketed for use in the treatment of GI nematode and cestode infections in horses. Most studies concerning praziquantel's mode of action have been done using the human trematode *Schistosoma mansoni* as a model parasite (Day et al., 1992). Praziquantel induces a rapid and sustained paralytic muscle contraction of the parasite and tegumental disruption. The contraction of parasite musculature is the primary effect, which is followed by a rapid vacuolization of the syncytial tegument. Muscular contraction and tegumental disruption are followed by exposure of parasite antigens, binding and penetration of host immune cells into the parasite (Martin, 1997). Metabolic changes include decrease in glucose uptake, glycogen storage, ATP content, and lactate release. All these effects are attributed either directly or indirectly to an alteration of intracellular Ca^{2+} homeostasis. Praziquantel effects are thought to be mediated by the release of intracellular stored Ca^{2+}, in addition to the increase of Ca^{2+} influx across the schistosome tegument (Day et al., 1992). Exposure and release of parasite antigens, as well as metabolic changes are attributable to the primary effect on the tegument. The exact molecular target, as well as its location within parasite tissues, remains to be completely understood. Several investigations have been focused on the beta subunit of voltage-gated Ca^{2+} channels as proposed targets for praziquantel action (Greenberg, 2005; Jeziorski and Greenberg, 2006). These channels are critical sites of extracellular Ca^{2+} influx and also play an important role in regulation of intracellular Ca^{2+} homeostasis. A variant of the beta subunit of voltage-gated Ca^{2+}channel was also found in platyhelminthes such as *Taenia solium* (Jeziorski and Greenberg, 2006). In addition, it has been demonstrated that praziquantel is an inhibitor of adenosine uptake and a relationship between this effect and praziquantel-mediated effects on Ca^{2+} influx has been hypothesized (Angelucci et al., 2007). Considering that platyhelminthes lack the biochemical pathway for purine biosynthesis, this mechanism may be involved on praziquantel antiparasitic activity. The chiral nature of praziquantel is other feature related to its pharmacological effects on the parasite. The drug has an asymmetric center in position 11b (see Figure 40.3) and, consequently, two enantiomeric forms: R(−) and S(+) praziquantel. Only the R(−) enantiomeric form has antiparasitic activity as shown by *in vitro* and *in vivo* experiments (Staudt et al., 1992; Cioli and Mattoccia, 2003; Angelucci et al., 2007).

Praziquantel can be formulated as tablet, paste, or suspension for oral administration, and as solution for SC or IM injections. Commercial formulations are racemates composed of equal parts of both praziquantel enantiomers. The pharmacokinetic behavior and metabolic fate of praziquantel has been investigated in sheep and dogs (Giorgi et al., 2001, 2003). These authors used a higher oral dose (30 mg/kg) than those recommended in these species in order to generate sufficiently high

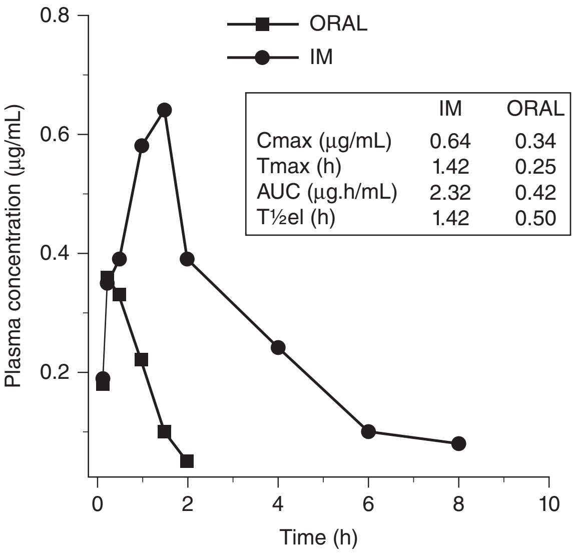


Figure 40.4 Praziquantel plasma concentration (µg/ml) profiles measured after its oral (30 mg/kg) and intramuscular (IM) (15 mg/kg) administrations to sheep. The inserted table shows mean pharmacokinetic parameters obtained following both treatments. C_{max}, peak plasma concentration; T_{max}, time to peak plasma concentration; AUC, area under concentration-time curve; T1/2el, elimination half-life. Source: Adapted from Giorgi et al., 2001. Reproduced with permission of John Wiley & Sons.

plasma concentrations for analysis. In sheep, praziquantel peak plasma concentration (C_{max}) and area under concentration-time curve (AUC) values administered by the IM route at 15 mg/kg were sixfold higher compared to those values observed following oral treatment at 30 mg/kg (Figure 40.4). The pharmacokinetics of praziquantel after its oral and IM administration was studied in water buffaloes, the major reservoirs for the human trematode *Schistosoma mansoni* in Asia (Sun and Bu, 2012); compared to the enteral administration, its relative bioavailability after the IM treatment was approximately 3.5-fold higher. It has been shown that praziquantel is completely absorbed in the GI tract in almost all species studied. Thus, its low oral bioavailability in sheep and water buffaloes is not attributed to poor GI absorption. An extensive hepatic first-pass effect accounts for the reduced praziquantel systemic availability. Liver oxidative metabolism of praziquantel in sheep resulted in the production of one hydroxylated metabolite, which might be either 11b-hydroxy- or 1-hydroxy-praziquantel (Giorgi et al., 2001). A cytochrome P450 3A isoenzyme was found to be involved in praziquantel hydroxylation in sheep liver. Conversely, the metabolite formed following praziquantel administration to dogs is 4′-hydroxy-praziquantel (Giorgi et al., 2003). This is consistent with previous studies in other species, such as rats, monkeys, and humans, in which 4′-hydroxy-praziquantel was the main metabolite (Masimirembwa and Hasler, 1994).

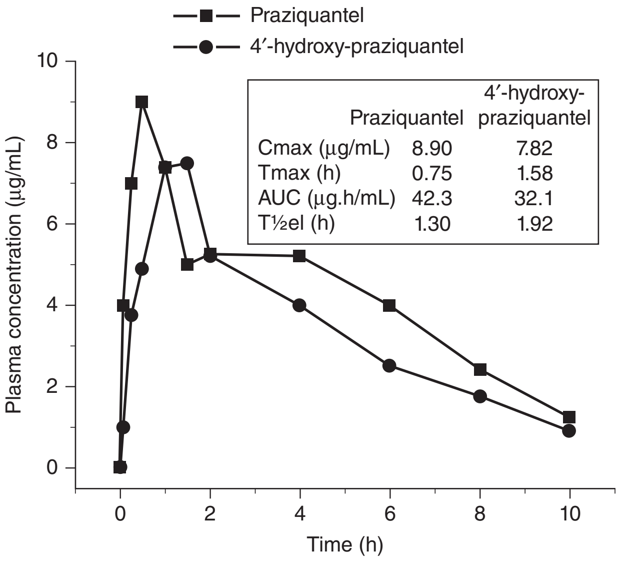


Figure 40.5 Plasma concentration profiles of praziquantel parent drug and its main hydroxylated metabolite (4′hydroxy-praziquantel) following praziquantel administration to dogs (orally, 30 mg/kg). The inserted table shows the comparative kinetic parameters for both molecules. C_{max}, peak plasma concentration; T_{max}, time to peak plasma concentration; AUC, area under concentration-time curve; T1/2el, elimination half-life. Source: Adapted from Giorgi et al., 2003. Reproduced with permission of Elsevier.

Praziquantel and its 4′-hydroxylated metabolite were depleted in parallel from plasma in dogs, which is supported by an AUC praziquantel/4′-hydroxy-praziquantel ratio equal to 1.3 (Figure 40.5). Increased levels of the parent drug and its metabolite following administration of liquid or dry grapefruit juice combined with praziquantel to dogs has been observed. It has been shown that grapefruit juice may be an inhibitor of the P-glycoprotein transport protein and cytochrome P450-mediated oxidative drug metabolism. Grapefruit juice may interfere with either the P-glycoprotein-mediated efflux or cytochrome P450-mediated oxidation of praziquantel. Interestingly, 4′hydroxy-praziquantel has been shown to exert roughly similar pharmacological activity compared to its parent compound (Staudt et al., 1992). Metabolism of praziquantel is also stereoselective for the two enantiomers. Thus, two 4′-hydroxy-praziquantel isomers were identified in the plasma of human volunteers (Westhoff and Blaschke, 1992). It has been shown that(−)4′-hydroxy-praziquantel is far more abundant in human plasma than its respective (+)isomer. This finding is highly relevant since, *in vitro*, there is no significant difference in the pharmacological effect of (−)praziquantel and its respective (−)4′hydroxylated isomer against *S. mansoni* (Staudt et al., 1992). It has been suggested that efficacy of praziquantel in humans is mainly due to the pharmacological activity of its (−)4′hydroxylated metabolite rather than to

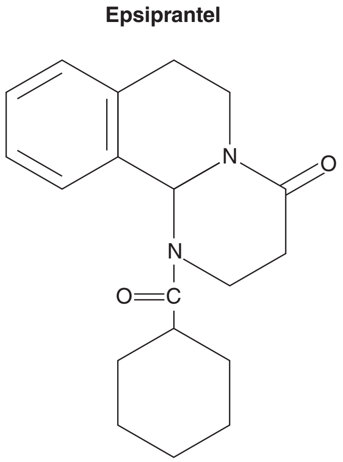


Figure 40.6 Epsiprantel chemical structure.

(−)praziquantel itself. Renal excretion is the main route of elimination of praziquantel and its metabolites.

Acute and chronic toxicity studies indicate a wide margin of safety for praziquantel. An acute, oral LD_{50} has not been established in dogs because they vomit when dosages exceed 200 mg/kg. The single therapeutic dose is 3.8–12.5 mg/kg in dogs and 4.2–12.7 mg/kg in cats. Overdoses of up to fivefold are tolerated without adverse effect. Studies in pregnant rats and rabbits detected no embryotoxic or teratogenic effects. Similar tests support the use of praziquantel in breeding and pregnant animals without restrictions.

Epsiprantel

This cestocidal compound (2-(cyclohexylcarbonyl)-oxo-1,2,3,4,6,7,8,12b-octahydropyrazino[2,1-a] [2] benzazepine) (Figure 40.6), is chemically related to praziquantel. Epsiprantel is used specifically for treatment of the common tapeworms of dogs (*D. caninum, T. pisiformis*) and cats (*D. caninum, T. taeniaeformis*). Recommended dosages are virtually 100% effective against those cestode parasites. Epsiprantel causes tegumental damage in larval and adult stages of *E. granulosus*. At a dosage of 5 mg/kg in dogs, the drug is 94% effective against immature (7-day-old) worms and more than 99% effective against mature stages (Thompson et al., 1991). At doses of 5.5 mg/kg in dogs and 2.5 mg/kg in cats, the drug was >99% effective against *E. multilocularis*, although residual worms may persist in some animals (Eckert et al., 2001). The recommended dose of 5.5 mg/kg is also 100% effective against *Taenia* spp. and 99.8% against *Dipylidium* (Corwin et al., 1989).

Similar to praziquantel, epsiprantel affects Ca^{2+} homeostasis within the parasite. The drug damages the tegument, making it vulnerable to lysis and digestion within the host's gut. Epsiprantel is only formulated for oral administration in dogs and cats. It is poorly absorbed in the GI tract and is thereby available to act against intestinal cestodes, being eliminated in feces. There are no metabolites and only 0.1% of the administered drug is eliminated by urine. The comparative pharmacological properties of praziquantel and epsiprantel are summarized in Figure 40.7.

Due to its low GI absorption, epsiprantel is a safe drug. For example, cats tolerate five times the therapeutic dose of this cestocide once daily for 3 consecutive days. Emesis has been reported in cats administered 40 times the therapeutic dose daily for 4 days. Beagle puppies (7–10 weeks of age) given 100 mg/kg once (18 times the recommended dosage) exhibited no signs of toxicity. Adult dogs treated once with a 36 times overdose were not adversely affected. Emesis is the most commonly observed side effect of epsiprantel and may occur in prolonged treatments.

Antitrematodal Drugs

Introduction

Fasciolosis, caused by the cosmopolitan liver fluke *Fasciola hepatica*, is the most common and economically important disease caused by trematode parasites in domestic animals worldwide. Fasciolosis is an important zoonotic disease, particularly in underdeveloped countries (Mas-Coma, 2004). Chemotherapy, based on the use of flukicidal compounds, is the main tool to control liver flukes. Most of the antitrematodal drugs discussed herein are compounds used for the treatment of fasciolosis. The tropical counterpart of *F. hepatica* is *F. gigantica* and all drugs active against one of the species are equally effective against the other (Boray, 1986). Rumen fluke (*Paramphistomum* spp.) infections are common in cattle and sheep throughout the world. Adult flukes attach to the rumen wall and are of little consequence to the health of the animal. Large numbers of the immature stages, however, can be seriously pathogenic as they migrate within the gut lumen from the duodenum to the rumen. Intestinal paramphistomosis generally responds well to treatment with drugs that are effective against liver fluke and/or cestode infections in ruminants. Readers interested on specific issues regarding treatment of infections caused by either rumen flukes (*Paramphistomum* spp.) in sheep and cattle or lung flukes (*Paragonimus* spp.) in dogs and cats, are referred to the previous edition of this textbook (see Reinemeyer and Courtney, 2001).

The liver fluke's life cycle has several features that should be considered for a better understanding of the pharmacological properties of the antitrematodal drugs. Both immature and mature flukes damage the host liver.

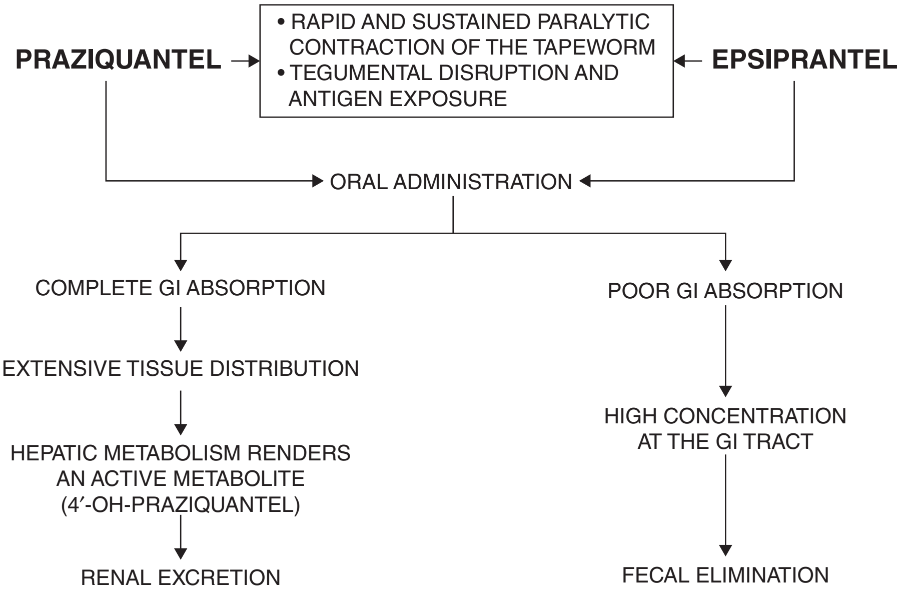

PRAZIQUANTEL
• RAPID AND SUSTAINED PARALYTIC CONTRACTION OF THE TAPEWORM
• TEGUMENTAL DISRUPTION AND ANTIGEN EXPOSURE
EPSIPRANTEL
ORAL ADMINISTRATION
COMPLETE GI ABSORPTION
EXTENSIVE TISSUE DISTRIBUTION
HEPATIC METABOLISM RENDERS AN ACTIVE METABOLITE (4′-OH-PRAZIQUANTEL)
RENAL EXCRETION
POOR GI ABSORPTION
HIGH CONCENTRATION AT THE GI TRACT
FECAL ELIMINATION

Figure 40.7 Comparative pharmacodynamic and pharmacokinetic properties of the chemically related anticestodal drugs: praziquantel and epsiprantel. GI, gastrointestinal tract.

After the infective metacercariae are ingested by grazing sheep or cattle, the immature fluke emerges from its cyst, penetrates the wall of the small intestine, traverses the peritoneal cavity, and penetrates the liver capsule within 4 days of infection. During the next several weeks, immature flukes tunnel through liver tissues, feeding and increasing rapidly in size. Acute and subacute fasciolosis are most common in sheep. Both are characterized by the migration of a high number of immature flukes through the liver parenchyma causing severe hepatic hemorrhage, jaundice, and anemia. These stages are often fatal. Clinical signs of acute and subacute fasciolosis are presented within 6–8 weeks postinfection.

Antitrematodal drugs used against immature forms must achieve effective concentrations within the liver tissue. The severity of *F. hepatica* infections in sheep may be enhanced by the fluke's potential role in disseminating enteric bacteria (*Clostridium novyi*) in the liver during migration (Reinemeyer and Courtney, 2001). During the eighth week of infection, flukes begin to penetrate the main bile ducts, where they attain sexual maturity by ~10–12 weeks after infection. Adult flukes are blood-sucking parasites and penetrate within the bile ducts, causing biliary hyperplasia and progressive occlusion. The infected areas may be surrounded by connective tissue, which progressively becomes calcified. Such type of tissue reaction is most common in cattle, were fasciolosis is typically more chronic and subclinical in nature. Although adult flukes are most susceptible (or perhaps most accessible) to fasciolicidal drugs at this stage, the affected areas of the liver tissue become progressively less penetrable by therapeutic agents and consequently more difficult to treat (Reinemeyer and Courtney, 2001).

Carbohydrate and energy metabolism in helminth parasites differs from those of the mammalian host. Also, parasites exhibit a wide variation in their carbohydrate break-down pathways during their life cycle (Kita et al., 1997). Parasite metabolism uses an aerobic–anaerobic transition. In general, free-living larval stages obtain their energy from aerobic processes, whereas adult forms have mainly an anaerobic metabolism. A transition in the pathways of energy metabolism was observed in both the cytosol and mitochondria of the liver fluke *F. hepatica* (Tielens et al., 1987; Tielens, 1994). Overall, energy metabolism in the adult stages is almost completely anaerobic, and aerobic processes remain only in the tegument of the parasite, which is also one of the main absorptive surfaces for drug uptake by the fluke and may be a primary target for anthelmintic action. Studies in *F. hepatica* have shown that the transtegumental transfer mechanism is critical to achieve sufficient drug concentration at the site of action to exert its flukicidal action (Mottier et al., 2006). Thus, adult forms in the bile ducts are exposed to anthelmintics or its metabolic products eliminated by bile excretion. Although the tegument represents only 1% of the total volume of the adult parasite, the integrity of the surface plasma membrane and the tegumental syncytium is essential for the viability of the fluke. The disruption of the tegument by different mechanisms (including those involved in energy generation) may have severe consequences for the parasite because the drug would penetrate deeper and disrupt also subtegumental tissues. The surface damage will be exacerbated by the surfactant action of the bile. In addition, adult blood-sucking stages may uptake the anthelmintic drugs through the alimentary canal. In fact, most of the fasciolicides have high efficacy against adult flukes due to their extended residence in the systemic circulation as a consequence of their strong plasma protein binding (~98–99%).

Table 40.1 Comparative efficacies of the most widely used antitrematodal compounds against different stages of the liver fluke *Fasciola hepatica*. Source: Fairweather 1999. Reproduced with permission of Elsevier.

<table>
<tr><th rowspan="2">Flukicide Drugs</th><th colspan="12">Fluke's Age (in Weeks)</th></tr>
<tr><th>1</th><th>2</th><th>3</th><th>4</th><th>5</th><th>6</th><th>7</th><th>8</th><th>9</th><th>10</th><th>11</th><th>12</th></tr>
<tr><td>DIAMPHENETIDE</td><td colspan="6">90–100%</td><td colspan="6">50–80%</td></tr>
<tr><td>TRICLABENDAZOLE</td><td></td><td colspan="2">90–99%</td><td colspan="9">99–100%</td></tr>
<tr><td>RAFOXANIDE</td><td colspan="3"></td><td colspan="3">50–90%</td><td colspan="6">91–99%</td></tr>
<tr><td>CLOSANTEL</td><td colspan="6"></td><td colspan="3">50–90%</td><td colspan="3">91–99%</td></tr>
<tr><td>NITROXYNIL</td><td colspan="6"></td><td colspan="3">50–90%</td><td colspan="3">91–99%</td></tr>
<tr><td>CLORSULON (oral)</td><td colspan="7"></td><td colspan="5">90–99%</td></tr>
<tr><td>CLORSULON (SC)</td><td colspan="9"></td><td colspan="3">70–99%</td></tr>
<tr><td>ALBENDAZOLE</td><td colspan="9"></td><td colspan="3">70–99%</td></tr>
<tr><td>OXYCLOZANIDE</td><td colspan="9"></td><td colspan="3">70–99%</td></tr>
</table>

Since the introduction of carbon tetrachloride for treatment of helminth infections of animals in the 1920s, numerous other compounds have been investigated for efficacy against *F. hepatica*. Carbon tetrachloride and other halogenated hydrocarbons have a narrow therapeutic index and their adverse effects (mainly hepatotoxicity) restrict their use for the treatment of fasciolosis. Based on their chemical structure, the fasciolicidal drugs can be classified into several groups:

1) *halogenated hydrocarbons* (carbon tetrachloride, hexachloroethane, tetrachlorodifluoroethane, hexachloroparaxylene);
2) *bisphenolic compounds* (hexachlorophene, bithionol), etc.;
3) *nitrophenolic compounds* (nitroxynil, disophenol, niclofolan);
4) *salicylanilides* (closantel, rafoxanide, oxyclozanide) and *brominated salicylanilides* (bromosalans);
5) *bencenesulfonamides* (clorsulon);
6) *benzimidazole* (albendazole, netobimin, luxabendazole) and *halogenated benzimidazole* (triclabendazole);
7) *phenoxyalkanes* (diamphenethide).

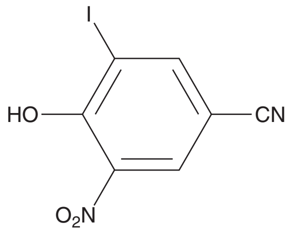
Most of the halogenated hydrocarbons, bisphenolic compounds, the nitrophenolics disophenol, and niclofolan, as well as the bromosalans, are old drugs not widely used today, and have been extensively reviewed in previous editions of this text (see Roberson and Courtney, 1995). This current chapter is focused on description of the pharmacological properties of the most currently used antitrematodal drugs. A schematic representation of the efficacy of different flukicidal drugs against immature and adult stages of *F. hepatica* is shown in Table 40.1.

Nitrophenolic Compounds

Nitroxynil

Nitroxynil (3-iodo-4-hydroxy-5-nitrobenzonitrile) (Figure 40.8) was developed in the late 1960s as an injectable fasciolicide for sheep and cattle. It is a trematodicidal compound highly effective against *F. hepatica*, which also holds activity against the abomasal nematode *Haemonchus contortus*. Nitroxynil is highly effective against adult stages of *F. hepatica* (from 8 weeks postinfection) and *F. gigantica*. Activity against earlier stages is rather erratic and it is not effective for the treatment of flukes younger than 6 weeks. Moreover, this anthelmintic is used for the control of ivermectin- and benzimidazole-resistant *H. contortus* in sheep. It is also marketed for the control of *Oesophagostomum* spp. and *Bunostomum* spp. in both sheep and cattle, and shows good activity against *Parafilaria bovicola* infections in cattle. On the contrary, nitroxynil is not effective for the treatment of paramphistomes in ruminants.

Due to the structural similarity between nitrophenols and 2,4-dinitrophenol, a known uncoupler of oxidative phosphorylation, it was assumed that nitroxynil and



Nitroxynil

Figure 40.8 Chemical structure of the nitrophenolic compound nitroxynil.

related compounds act in a similar way in the fluke. However, there is little direct evidence showing this mechanism of action for these fasciolicides. It has been shown that nitroxynil produced a rapid spastic paralysis (within 3 hours) of the fluke *in vitro* at similar concentrations to those found in the systemic circulation *in vivo* (Fairweather et al., 1984). This action was not believed to be consequence of an uncoupling effect on the oxidative phosphorylation (Fairweather and Boray, 1999). More recently, it was shown that nitroxynil causes severe disruption of the tegument of *F. hepatica*, both *in vitro* and *in vivo* (McKinstry et al., 2003). Liver flukes recovered from rats after 24 hours of nitroxynil treatment showed extensive swelling and blebbing of the tegument in both dorsal and ventral surfaces. These changes became more severe after 48 and 72 hours following nitroxynil administration and specimens recovered following these posttreatment intervals showed more widespread tegumental loss. Although the intrinsic mechanism of nitroxynil action on the parasite has not been established, it was shown that the tegument is an important target for this flukicidal drug.

Although nitroxynil can be administered orally or intraruminally (IR), it is more effective when administered by parenteral routes (SC, IM). A ruminal microflora-mediated nitroreduction may account for its low efficacy after oral or IR treatments. Solutions (25% or 34%) of nitroxynil-*N*-alkylglucamine are available in the market to be used at 10 mg/kg. SC and IM administrations provide similar efficacy against liver flukes, but the SC route has become the method of choice in practice. Local tolerance at the site of injection is satisfactory, although transitory inflammatory swellings are occasionally observed in cattle. Formulations containing nitroxynil plus ivermectin or nitroxynil in combination with ivermectin and clorsulon are marketed for SC administration in ruminants. Nitroxynil is well and rapidly absorbed after its SC administration and binds strongly to plasma proteins (~98%), mainly to albumin (Alvinerie et al., 1991). Plasma concentrations in sheep remain high within 3 days posttreatment and may be detected in the bloodstream for 60 days. Age and/or body weight-related differences on the pharmacokinetic behavior of nitroxynil were demonstrated in sheep (Moreno et al., 2010). These authors observed lower nitroxynil AUC and mean residence time (MRT) values in lambs (6–8 months old) compared to older sheep (18–20 months old). Due to its high affinity for plasma proteins, nitroxynil has low tissue distribution and, consequently, the amount of drug reaching the liver parenchyma seems to be insufficient for treatment of immature flukes at the recommended dose rate.

Nitroxynil was shown to be metabolized by hydrolysis of the cyano group to produce 3-iodo-4-hydroxy-5-nitrobenzamide and 3-iodo-4-hydroxy-5-nitrobenzoic acid in rat liver. The enzyme(s) responsible for this hydrolytic reactions was primarily localized in the cytosol (Markus and Kwon, 1994). Nitroxynil was also found to be nitroreduced in rat liver into 3-iodo-4-hydroxy-5-aminobenzonitrile (Maffei Facino et al., 1982). Further methylation of this metabolite into 3-iodo-4-methoxy-5-aminobenzonitrile was also shown. However, the inactive metabolites were not detected in the urine nor in the plasma of nitroxynil-treated sheep. Indeed, unchanged drug is likely to be the only source responsible for nitroxynil action. Due to its high plasma protein binding, it was suggested that the blood is the primary source of drug uptake via the fluke's digestive system. In addition, the levels of nitroxynil in bile are far lower than those observed in plasma, and so the uptake of this anthelmintic through the tegument would be limited (McKinstry et al., 2003). The urine is the primary route of nitroxynil elimination, although the drug is also excreted in feces and milk (it is not approved for use in dairy animals).

Animals can be slaughtered for human consumption 60 days after nitroxynil treatment. Nitroxynil also stains wool or hair yellow; thus, care must be exercised to avoid spilling. The drug is well tolerated in cattle and sheep at the therapeutic dose of 10 mg/kg. Nitroxynil side effects in these species include hyperthermia and hyperpnoea, which were associated with the uncoupling of oxidative phosphorylation in the target species and observed at higher dose levels. Doses above 40 mg/kg may be lethal.

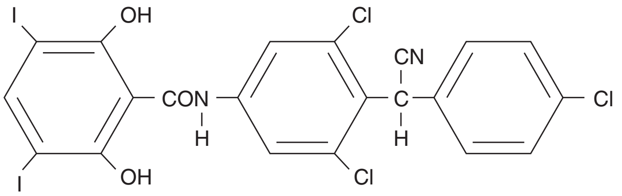
Other nitrophenolic flukicidal compounds such as disophenol and niclofolan were replaced by nitroxynil and many other newer and safer fasciolicidal drugs.

Salicylanilides

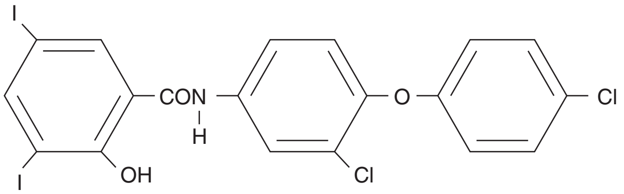
This family includes fasciolicidal drugs of similar chemical structure sharing the same mode of action. The most extensively used salicylanilides (closantel, rafoxanide, and oxyclozanide) are described here. For information on the flukicidal activity of the brominated salicylanilides (bromosdalans) readers are referred to a previous edition of this text (Roberson and Courtney, 1995).

Closantel

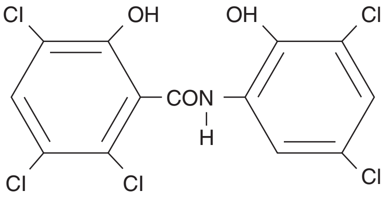
Closantel (*N*-[5-chloro-4-[(4-chlorophenyl) cyanomethyl]-2-methylphenyl]-2-hydroxy-3,5-diiodobenzamide) (Figure 40.9) is highly effective for the treatment of adult flukes and shows good activity against immature specimens aged 6–8 weeks (Table 40.1). It is not effective against earlier stages (Boray, 1986). At an oral dosage of 10 mg/kg, its efficacy is more than 92% against 8-week-old and adult *F. hepatica*. It is less active against younger stages of this parasite, that is 70–77% efficacy for 6-week-old flukes migrating in the liver. It is also effective (94.6–97.7%) against 8-week-old *Fascioloides*



Closantel



Rafoxanide



Oxyclozanide

Figure 40.9 Chemical structures of salicylanilide flukicidal compounds: closantel, oxyclozanide, and rafoxanide.

magna in sheep at oral dosages of 15 mg/kg or IM dosages of 7.5 mg/kg. However, closantel is not effective against paramphistome flukes.

Closantel is also highly effective against *H. contortus* in sheep and is used as an alternative drug for the treatment of ivermectin-, benzimidazole-, levamisole-, and morantel-resistant strains of this nematode. In addition, it is effective for the treatment of many other adult nematodes such as *Oesophagostomum* spp., *Bunostomum* spp., and *Ostertagia* spp., in both sheep and cattle. Additionally, closantel is effective against certain ectoparasites such as blood sucking lice, ticks, mites, and certain grubs of ruminants. It is also used for the treatment of the nasal bot *Oestrus ovis* in sheep. The drug can be used against the adult stage of *Ancylostoma caninum*, but is not effective against the somatic larvae of this hookworm. Closantel also has been used in horses to prevent or reduce infections of *Strongylus vulgaris* and *Gasterophilus* spp.

Closantel and other salicylanilides are uncouplers of the oxidative phosphorylation in the liver fluke. There is stronger evidence supporting this mechanism of action for these compounds than for nitrophenolic flukicides (Martin, 1997). ATP production in the mitochondria is coupled to a proton (H^+) gradient across the inner mitochondrial membrane. During the oxidative phosphorylation, electrons coming from NADH or FADH are transported through a series of protein complexes on the inner mitochondrial membrane. During this process, protons (H^+) are pumped out of the mitochondrial matrix into the space between the inner and outer mitochondrial membrane, establishing a proton gradient across the inner membrane. This proton motive force is due to the pH gradient and transmembrane electric potential being critical for ATP synthesis. Salicylanilides possesses a detachable proton (H^+) which could be shuttled across the inner mitochondrial membrane, removing the proton (H^+) gradient and uncoupling oxidative phosphorylation. Metabolic changes consequently to this mechanism include: increase in glucose uptake, decrease in glycogen content, changes in respiratory intermediates, and decrease in ATP synthesis (Fairweather and Boray, 1999).

In addition, an alternative mechanism of action of closantel has been investigated. A significant decrease in tegumental pH (6.8 to 6.5) was observed after its *in vitro* incubation with *Schistosoma mansoni* and *F. hepatica* specimens (Pax and Bennett, 1989). In fact, this fall occurred within 10 minutes after closantel addition and before any change in the production of ATP by the parasite. This effect was also observed at a lower concentration than that required to produce any reduction in ATP synthesis. It was concluded that the action of closantel is more complex than a conventional uncoupler of oxidative phosphorylation. Indeed, this anthelmintic is a membrane-active molecule capable of affecting many biochemical and physiological processes within the parasite. Closantel may also shuttle protons (H^+) across the tegumental membrane leading to a disruption of the mechanisms responsible for maintaining pH homeostasis, which are likely to reside in the tegument and excretory system (Fairweather and Boray, 1999). Closantel also induces spastic paralysis in *F. hepatica* at concentrations comparable to maximum blood levels attained *in vivo*. This effect was attributed to an increase in calcium ions in the muscle cells. It has been suggested that this neuromuscular action of closantel may take place before any disruption of energy metabolism. Consequences of the paralysis are cessation of feeding and starvation; the fluke has to draw on its energy reserves to survive and this may account, at least in part, for some of the biochemical changes observed.

Closantel is a weakly acidic molecule (pKa = 4.28) of high molecular weight (663 KDa) and extremely lipophylic. It is formulated for oral, IR, or parenteral (SC or IM) administration in ruminants. Closantel is marketed as 3.75 or 5% suspensions or solutions for oral drench or IR administration (solutions also may be used for parenteral administration). An injectable (SC) formulation of closantel and ivermectin has been licensed for use in cattle. The pharmacokinetic behavior of both anthelmintics administered as a combined product has been studied in this species (Cromie et al., 2006). The plasma availability of both active drugs in the combination was not affected by the presence of the other. Plasma profiles of closantel in the combined product were similar to those observed after administration of a reference product. On the contrary, ivermectin was absorbed and eliminated faster following its administration in the combined formulation compared with a reference product containing only ivermectin.

Closantel is well absorbed after both enteral or parenteral administrations in sheep and cattle. The recommended enteral dose rate in these species is 10 mg/kg whilst the same efficacy could be attained following its SC or IM administration at 5 mg/kg. Consequently, the oral bioavailability of closantel administered at 10 mg/kg was similar to that observed following its parenteral administration at 5 mg/kg (Michiels et al., 1987). It was shown that the bioavailability of closantel administered by the oral route was 50% lower compared to that observed following its parenteral administration. It has been shown that closantel is not subjected to any significant biodegradation by the ruminal fluid, but more than 80% of the drug was shown to be associated with the particulate phase of the abomasal digesta (Hennessy and Ali, 1997). The strong association of closantel with the particulate digesta, and the limited time available for the exchange of the drug between the particulate material and the GI fluids prior to absorption, may explain its low enteral bioavailability (Hennessy and Ali, 1997). In addition, since closantel is a weak acid, almost 99.5% of the total drug present in the intestinal lumen may be in the ionized form at the absorption site in the intestine. Altogether these observations may help to explain the incomplete GI absorption of closantel after its oral administration in ruminants. A reduction in feed intake slows the digesta flow rate and extends the residence of the drug within the GI's lumen, which accounted for increased closantel plasma and GI availabilities in sheep (Hennessy and Ali, 1997).

Closantel is extensively bound to plasma proteins (>99%), mainly albumin, and has a long terminal half-life of 14.3–14.5 days in sheep (Ali and Bogan, 1987; Hennessy, 1993). It binds specifically to ovine serum albumin at site I, the warfarin/phenylbutazone binding site of albumin, and also to invertebrate hemocyanin and hemolymph (Rothwell et al., 2000). As consequence of its high protein binding, the duration of therapeutic levels of closantel in plasma is prolonged. Thus, a single dose of closantel protects sheep against susceptible *H. contortus* reinfection for up 28 days (Hall et al., 1981). A small apparent volume of distribution (Vd) (<0.15 l/kg) indicates that the distribution of closantel to tissues (including the liver) is limited in ruminant species (Michiels et al., 1987; Swan et al., 1999). Overall, plasma albumin constitutes a reservoir from which this anthelmintic is directly available to hematophagous helminthes such as *F. hepatica* and *H. contortus*. Indeed, the blood is the primary source for the uptake of closantel via the digestive system of the parasites.

Dehalogenation in the liver renders two inactive monoiodo metabolites (3- and 5-monoiodoclosantel), although the metabolism of closantel is poor. Approximately 80–90% of the administered drug is eliminated by biliar/fecal excretion without metabolic changes. Only 10% of the administered drug was eliminated as monoiodo metabolites in sheep. Other minor routes of elimination of closantel are urine (less than 0.5% of the administered dose) and milk. In dairy cattle, approximately 1% of the administered dose was found to be excreted with the milk per day. Residual tissue concentrations are extremely low in sheep: seven to 21 times lower than the corresponding plasma concentrations. Animals may be slaughtered for human consumption 28 days after closantel treatment. In cattle, closantel concentrations in plasma were higher than those observed in milk. Similarly, mean plasma C_{max} and AUC values resulted approximately 44- and 66-fold higher, respectively, compared to those observed in milk after the oral administration of closantel (10 mg/kg) to dairy goats (Iezzi et al., 2014) (Figure 40.10). The total amount of closantel recovered in milk was equal to 1.7% of the administered dose. In this species, the calculated withdrawal time for closantel in milk was between 39 and 43 days postadministration. Moreover, closantel residual concentrations in milk subproducts, such as cheese and ricotta, were higher than those measured in the milk used for their production. For

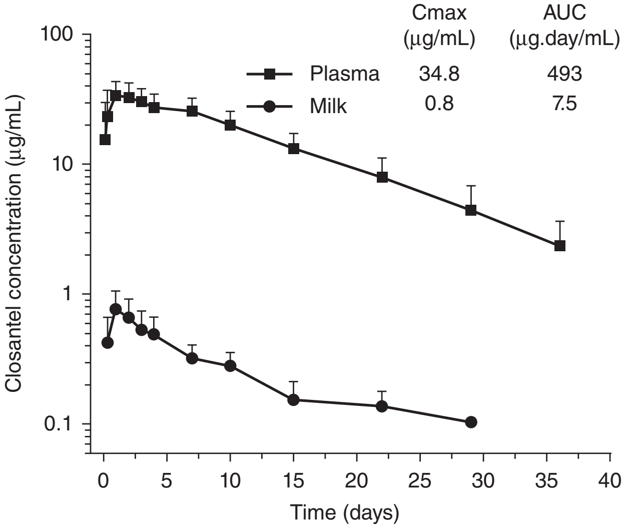


Figure 40.10 Comparative plasma and milk concentration profiles of closantel measured after its oral administration (10 mg/kg) to dairy goats. The insert shows mean closantel C_{max} and AUC values both in plasma and milk. C_{max}, peak plasma concentration; AUC, area under the concentration-time curve. Source: Adapted from Iezzi et al., 2014. Reproduced with permission of John Wiley & Sons.

these reasons, the use of this anthelmintic in dairy animals is forbidden.

The acute toxicity resulting from single dosing of closantel in rats is characterized by hypotonia, ataxia, diarrhea, and dyspnea. Clinical signs of acute toxicity in ruminants are anorexia, labored breathing, general weakness, and decreased vision (or blindness), appearing approximately 1 week after dosing. At the lethal dose (LD_{50} in sheep = >40 mg/kg oral/IM; LD_{50} in cattle = >40 mg/kg oral and >20 mg/kg IM), anorexia, hypotonia, and quadriplegia preceded death. In short-term toxicological studies conducted in rats, male gonads and the liver were the target organs for toxicity. Some fatty deposition was observed in the liver tissue of males but not in females. Extensive reproductive studies in rams, ewes, and bulls indicate that closantel has no risk to reproductive parameters.

Rafoxanide

This anthelmintic is an halogenated salicylanilide. Its chemical formula is 3′-chloro-4′-(p-chlorophenoxy)-3,5-diiodosalicylanilide (Figure 40.9). It is an off-white crystalline powder and is commercially formulated for use as a bolus or as a drenching suspension, which may be also used for IR administration. Rafoxanide was developed in 1969 and subsequently has been used extensively worldwide against fasciolosis and haemonchosis in sheep and cattle. Its principal use is as an adulticide for *F. hepatica* and *F. gigantica*. A single therapeutic dose (7.5 mg/kg) in sheep provides nearly 100% for 12-week-old flukes of *F. hepatica*, 86–99% for 6-week-old flukes, and 50–98% for 4-week-old specimens of this parasite. At elevated doses (10–15 mg/kg), rafoxanide shows high activity against 4-week-old flukes (Boray, 1986). The same dosages reach similar efficacies against *F. hepatica* in cattle. The reliable efficacy of this drug against 4- and 6-week-old flukes gives rafoxanide an advantage over strictly adulticidal drugs in the treatment of acute fasciolosis. In this case, the administration might be repeated after 3 weeks to eliminate maturing flukes that may have escaped the earlier treatment. In addition, this anthelmintic is also indicated for the treatment of haemonchosis, bunostomosis, and sheep nasal bots (*Oestrus ovis*). The mode of action of rafoxanide is similar to that described for the chemically related salicylanilide closantel.

Following oral dosing, rafoxanide is absorbed from the small intestine into the bloodstream. Peak plasma levels occur between 24 and 48 hours. Its bioavailability, measured as AUC and C_{max}, was 2.5 to 3-fold greater in 5 to 8-week-old suckling lambs compared to weaned lambs aged 21–22 weeks (Swan and Mülders, 1993). Rafoxanide is extensively bound (>99%) to plasma proteins and has a long (16.6 days) terminal half-life. The blood is the primary source for the uptake of rafoxanide via the digestive system of the hematophagous helminths. Also, the efficacy of rafoxanide against immature liver flukes is due to its prolonged persistence in the plasma, with subsequent effects on maturing flukes as they reach the bile ducts. In addition, liver fluke infection did not alter the plasma disposition of rafoxanide in sheep (Benchaoui and McKellar, 1993). An injectable solution of ivermectin (1%) plus rafoxanide (12.5%) has been licensed for the treatment of endo- and ectoparasites of cattle, buffaloes, camels, sheep, and goats (El-Banna et al., 2008). This combined formulation is available for SC injection at 2.5 mg/kg of rafoxanide and 200 µg/kg of ivermectin. Enhanced ivermectin AUCs and prolonged elimination half-lives were observed after administration of this combined product to both adult male sheep and calves. Whether or not rafoxanide inhibited the P-glycoprotein-mediated elimination of ivermectin remains to be elucidated.

Rafoxanide is not metabolized to any detectable degree by cattle or sheep liver. Following a single oral dose of 15 mg/kg in cattle, no residue of the compound is detectable in edible tissues at 28 days after treatment. Interestingly, approximately a 20% loss of rafoxanide residues was observed in both fried and roast cattle beef (Cooper et al., 2011). Rafoxanide is not allowed for treating animals whose milk is intended for human consumption. Neither pasteurization nor manufacturing processes for production of skim-milk powder affected the stability of rafoxanide residues (Power et al., 2013). Moreover, rafoxanide residues were between 10- and 14-fold higher in skim-milk powder, butter, and cheese than in the starting milk.

Oxyclozanide

Oxyclozanide (3,3′,5,5′,6-pentachloro-2′-hydroxy salicylanilide) (Figure 40.9) was introduced over 40 years ago for use against adult fluke infections in ruminants. It has been also used against tapeworms (*Moniezia* spp.) in sheep and cattle. This anthelmintic is marketed as an oral drench (aqueous suspension) containing oxyclozanide only or in combination with levamisole hydrochloride or oxfendazole. It is also formulated as a powder to be incorporated in the feed. The recommended dose rates are 10–15 mg/kg for cattle and 15 mg/kg for sheep and goats. The mechanism of action of oxyclozanide is similar to that described for closantel. Following its oral administration to sheep, the drug is extensively (greater than 99%) bound to plasma proteins and the plasma concentration/time curve could be best described by a triexponential equation. Its terminal half-life (6.4 days) is shorter compared with closantel and rafoxanide (14.5 and 16.6 days, respectively) (Ali and Bogan, 1987). The drug is excreted into the bile as a glucuronide metabolite. Figure 40.11 shows the comparative plasma profiles and some kinetic parameters for closantel, rafoxanide, and oxyclozanide following their respective oral administrations to sheep.

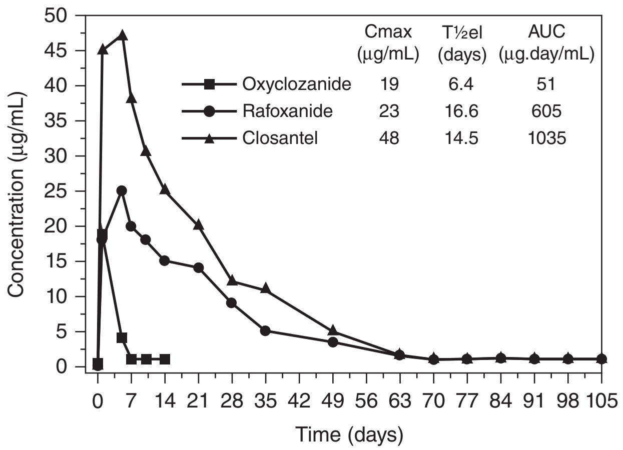
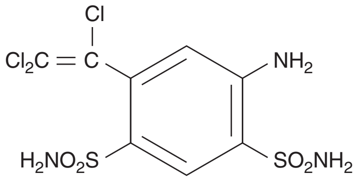


Figure 40.11 Comparative plasma profiles of closantel (7.5 mg/kg), rafoxanide (7.5 mg/kg), and oxyclozanide (15 mg/kg) measured after their oral administration to sheep. C_{max}, peak plasma concentration; T1/2el, elimination half-life; AUC, area under the concentration-time curve. Source: Adapted from Ali and Bogan, 1987. Reproduced with permission of John Wiley & Sons.

Benzenesulfonamides

Clorsulon

This anthelmintic is a benzenesulfonamide with the chemical formula 4-amino-6-trichloroethenyl-1,3-benzenedisulfonamide (Figure 40.12). Clorsulon is highly effective against adult liver flukes in sheep and cattle. However, is more effective in cattle than in sheep and goats. The recommended dose rate is 2 mg/kg body weight given by SC injection. Good efficacy against 8-week-old flukes is achieved following SC administration at 4–8 mg/kg. Oral administration at 3.75 mg/kg is effective for the treatment of adult liver flukes aged 14–16 weeks in both sheep and cattle. The recommended oral dose rate is 7 mg/kg, which is 100% effective for the treatment of 8-week-old flukes. The same dose rate is 99% effective for the treatment of adult specimens (14 weeks postinfection) in goats. The efficacy of clorsulon has been tested against infections with several other fluke species in ruminants. It was effective (>92%) against immature (8-week-old) *Fascioloides magna* in cattle and sheep at 21 mg/kg orally.



Clorsulon

Figure 40.12 Chemical structure of clorsulon, a bencenesulfonamide flukicidal compound.

Daily dosing at 7 mg/kg for 5 consecutive days has been 100% effective against adult and 92% effective against immature *Fasciola gigantica* in cattle. Clorsulon has poor efficacy against *Paramphistomum* spp.

The combination of clorsulon with ivermectin in a SC injectable formulation was designed for simultaneous treatment of *F. hepatica* and nematode infections of cattle. The oral formulation of clorsulon also can be used concurrently with other anthelmintics (e.g., ivermectin, fenbendazole) with no reduction in efficacy of the individual products. An injectable combined flukicidal and nematodicidal formulation containing nitroxynil, clorsulon, and ivermectin was shown to be highly effective against 2 and 4-week-old early immature flukes (Hutchinson et al., 2009).

Chemically, clorsulon is similar to 1,3 diphosphoglycerate, an intermediary product of glycolysis. Consequently, this anthelmintic was shown to inhibit competitively the enzymes phosphoglycerate kinase and phosphoglyceromutase (Schulman and Valentino, 1982). However, such disruption of glycolysis was observed *in vitro* with clorsulon concentrations many times greater than blood levels achieved *in vivo*. Thus, it is believed that clorsulon effects on the glycolytic pathway at therapeutic concentrations attained in vivo may not be relevant. Nevertheless, glycolysis is the main energy yielding pathway in the fluke and any disruption of this process would decrease glucose utilization and the production of metabolic end products (acetate and propionate) with a significant reduction in ATP levels within the parasite. Morphological changes in fluke specimens recovered from clorsulon-treated rats include severe necrosis of the gastrodermal cells and tegumental damage restricted to the cone and midbody regions of the fluke (Fairweather and Boray, 1999; Meaney et al., 2003, 2004). It has also been shown that the gut was more severely affected than the tegument, which is consistent with the observation that oral ingestion is a main route of entry of clorsulon into the fluke (Meaney et al., 2005). Thus, the changes in the gut represent the direct consequence of drug action, while those observed in the tegument represent either the direct effect of transtegumental uptake of clorsulon from the bile or an indirect effect derived from gut disruption. Clorsulon in combination with triclabendazole caused a greater disruption of the fluke's tegument compared to the individual drugs (Meaney et al., 2005). This effect was observed in vitro and in vivo when the combination was used against a triclabendazole-resistant strain of *Fasciola hepatica*.

Clorsulon is marketed as an oral drench for sheep and cattle and as an injectable formulation (in combination with ivermectin) for SC administration to cattle. The recommended dosage of clorsulon for cattle and sheep is 7 mg/kg by oral drench. In commercial combination with ivermectin, clorsulon is administered SC to cattle at a rate of 2 mg/kg and ivermectin at 0.2 mg/kg. The oral drench is an aqueous suspension containing 8.5 mg/ml of clorsulon dissolved in propylene glycol/water (1/10). The pharmacokinetic behavior of clorsulon was studied in sheep and goats after administration of a single IV and after a single oral dose of 7 mg/kg (Sundlof and Whitlock, 1992). These authors reported that the bioavailability of orally administered clorsulon was approximately 55% in goats and 60% in sheep. Peak plasma concentrations occurred at 14 hours and 15 hours after oral administration in goats and sheep, respectively. Absorption from the GI tract effectively prolonged the elimination of clorsulon, increasing plasma elimination half-lives (about twofold) and MRTs (approximately three to fourfold) in both sheep and goats. It has been shown that the rumen may substantially influence the absorption pattern and the resultant pharmacokinetic behavior and antiparasite activity of enterally delivered anthelmintic drugs. This fact has been thoroughly studied for benzimidazole anthelmintic drugs. Similarly, when a suspension of clorsulon is deposited in the rumen, the adsorption of its particles to digesta solid content, the slow mixing and long digesta residence time, and the large rumen volume assists absorption by delaying the rate of passage of drug down the GI tract (Hennessy, 1993). Thus, the rumen may act as a reservoir and prolong the duration of drug absorption and/or outflow down the GI tract. Indeed, the absorption pattern of clorsulon from the GI tract influences its pharmacokinetic behavior and, consequently, its elimination from plasma is delayed. In rats, clorsulon was shown to be bound predominately to erythrocytes at doses below 4 mg/kg. In fact, the drug is bound to red blood cell carbonic anhydrase (Schulman et al., 1982). As explained above in this section, this is the major route of entry of clorsulon into the fluke and drug uptake by *F. hepatica* increase in direct proportion to the blood level.

Renal excretion of the parent drug is thought to be the main route of elimination of clorsulon, accounting for approximately 50% of the IV dose at 48 hours in both sheep and goats. On the other hand, up to 30% and 41% of the orally administered dose was recovered in urine in sheep and goats, respectively (Sundlof and Whitlock, 1992). The elimination rate constant (kel) in goats was nearly twice as large as the value determined in sheep, and the AUC following IV administration in goats was only 63% of the value observed in sheep, indicating that goats are more effective in their ability to eliminate clorsulon than are sheep. These differences in drug disposition between sheep and goats may account for the reduced efficacy of clorsulon reported in goats.

Clorsulon is a safe drug with high therapeutic index. Acute toxicity of this antiparasite has been assessed in mice, rats, sheep, and cattle. The LD_{5O} in mice is an intraperitoneal (IP) dose of 761 mg/kg and more than 10,000 mg/kg orally. The latter dose causes no apparent toxicity in rats. Sheep infected with flukes have been dosed repeatedly with 5 mg/kg daily for 28 days or with single doses of 100 mg/kg with no apparent effect. Uninfected sheep tolerated 200 or 400 mg/kg without adverse reactions. A toxic dosage has not been identified in cattle. Oral doses of 7 mg (recommended) and 21 mg/kg on 3 consecutive days and single oral doses of 7, 70, and 175 mg/kg (i.e., up to 25 times the label dosage) have not affected weight gains, feed consumption, or clinical or histopathological parameters adversely. In uninfected goats, experimental dosages up to 35 mg/kg every other day for three doses did not cause adverse reactions. Clorsulon is also considered safe for use in breeding and pregnant animals. Residue studies indicate a short half-life of clorsulon in tissues and milk. Milk taken from treated animals within 72 hours (six milkings) after treatment should not be used for human consumption, and beef animals should not be slaughtered within 8 days of treatment.

Benzimidazoles

The pharmacology of the broad-spectrum benzimidazole (BZD) anthelmintic compounds has been fully described in Chapter 39. Albendazole (used at 7.5 mg/kg in sheep and at 10 mg/kg in cattle) and the pro-BZD netobimin (20 mg/kg) are active against liver flukes older than 12 weeks. Fenbendazole is not effective against *F. hepatica*, but a single treatment at 5 mg/kg reduced *F. gigantica* infection in sheep by up to 95%. Luxabendazole is also an effective flukicidal BZD which did not reach the market. However, the halogenated BZD thiol named triclabendazole, which does not have nematocidal activity, is the most widely used and potent flukicidal drug active against immature and mature stages of *F. hepatica*. Its pharmacological features are described in Section Triclabendazole.

Triclabendazole

Unlike other BZD compounds, the halogenated derivative triclabendazole (5-chloro-6(2-3 dichlorophenoxy)-2-methylthio-1H-benzimidazole) (TCBZ) (Figure 40.13) holds an excellent efficacy against the adult and juvenile stages of *F. hepatica* (down to 1-week-old flukes), which is unlike other available flukicidal drugs (Boray et al., 1983). The high activity of TCBZ against immature flukes is significant because they represent the migratory stage of the parasite, which may produce serious tissue damage in the host. However, TCBZ activity appears to be restricted to *F. hepatica*, *F. gigantica*, *Fascioloides magna*, and the lung fluke, *Paragonimus* spp. (Calvopiña et al., 1988), because the drug does not show clinical efficacy against nematodes, cestodes, and other trematode (*Dicrocoelium dendriticum*, *Paramphistomun* spp., and *Schistosoma mansoni*) parasites.

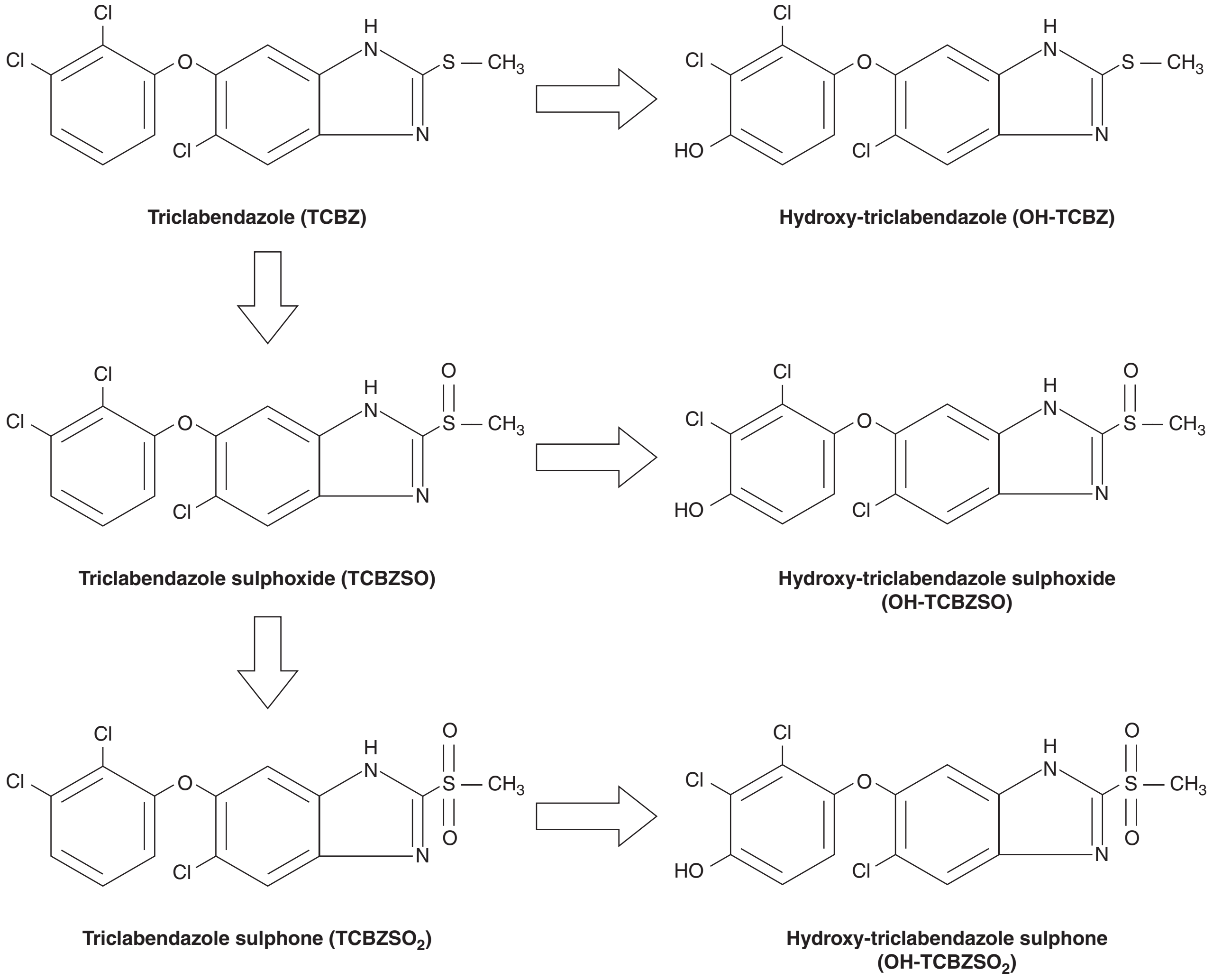

Triclabendazole (TCBZ)
Hydroxy-triclabendazole (OH-TCBZ)
Triclabendazole sulphoxide (TCBZSO)
Hydroxy-triclabendazole sulphoxide (OH-TCBZSO)
Triclabendazole sulphone (TCBZSO$_2$)
Hydroxy-triclabendazole sulphone (OH-TCBZSO$_2$)

Figure 40.13 Triclabendazole (TCBZ) and its main metabolites: chemical structures and metabolic pathways. See the text for further explanation on TCBZ metabolism.

The precise mode of action of TCBZ has not yet been established. BZD nematocidal activity is based on its binding to parasite β-tubulin (Lacey, 1988), which inhibits polymerization into microtubules. Thus, all the functions ascribed to microtubules at the cellular level are altered (cell division, maintenance of cell shape, cell motility, cellular secretion, nutrient absorption, and intracellular transport). BZD methylcarbamate molecules such as albendazole or fenbendazole act upon nematode microtubules at the tubulin colchicine binding site (Lacey, 1988). It is likely that a different site of action is involved on the flukicidal activity of TCBZ, which could also explain its lack of efficacy against other helminth parasites. However, there are morphological data that support the possible binding of TCBZ to the fluke's tubulin. The TCBZ sulfoxide metabolite (TCBZSO) blocks the transport of secretory bodies from the cell body to the tegumental surface, culminating in the total loss of the tegument (Stitt and Fairweather, 1994). TCBZSO also inhibits the mitotic division of spermatogenic cells and the stem vitelline cells, all changes related to microtubule inhibition. It is likely that TCBZ/metabolites may target an alternative binding site on the tubulin molecule (Fairweather, 2005).

The pharmacokinetics of TCBZ has been characterized in sheep (Hennessy et al., 1987), goats (Kinabo and Bogan, 1988), and cattle (Sanyal, 1995). TCBZ parent drug is not detectable in plasma after its oral administration to sheep and cattle, TCBZSO and TCBZ-sulfone (TCBZSO$_2$) being the metabolites recovered from the bloodstream of treated animals (see Figure 40.14). These metabolites reach a peak plasma concentration after 18 and 36 hours posttreatment, respectively, in sheep. Furthermore, extremely low concentrations of TCBZ were recovered in bile, TCBZSO, TCBZSO$_2$, and the hydroxy-TCBZ derivatives being the major

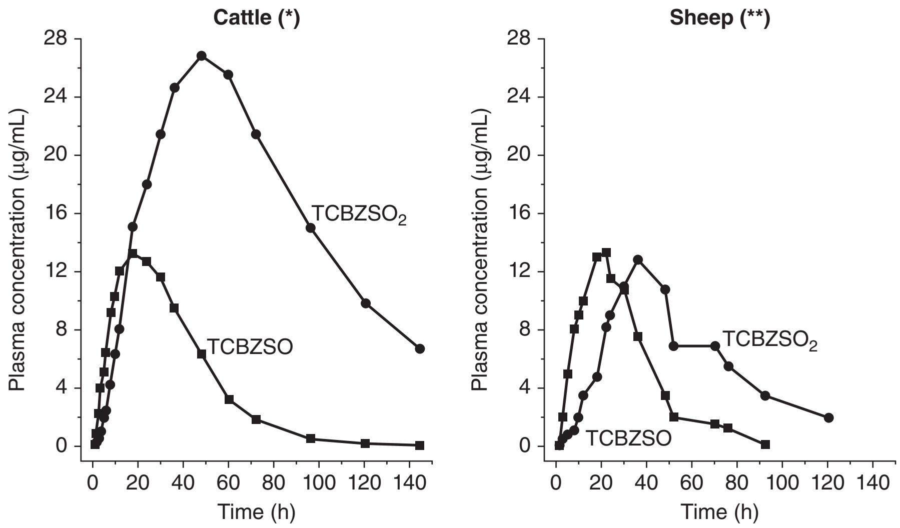


Figure 40.14 Plasma concentration profiles of triclabendazole sulfoxide (TCBZSO) and sulfone (TCBZSO$_2$) obtained after triclabendazole administration to cattle (orally, 12 mg/kg) and sheep (intraruminal, 10 mg/kg). Source: *Data from Lanusse et al., unpublished observations. **Adapted from Hennessy et al., 1987.

biliary metabolites found in sheep (Hennessy et al., 1987) (see Figure 40.13). The metabolic fate of TCBZ and its metabolites was characterized in sheep (Virkel et al., 2006). Both flavin monooxygenase (FMO) and cytochrome P450 enzymatic systems were found to be involved in the sulfoxidation and sulfonation of TCBZ in sheep liver. In addition, sheep ruminal microflora was able to reduce TCBZ sulfoxide derivatives, TCBZSO and hydroxy-TCBZSO, into TCBZ and hydroxy-TCBZ, respectively.

As for other nematocidal BZD sulfoxides, the sulfoxide metabolite of TCBZ is believed to have flukicidal activity. In fact, the tegument of *F. hepatica* is highly susceptible to TCBZSO, since the compound causes severe surface damage after a relatively short exposure time (Stitt and Fairweather, 1994). TCBZ metabolites bind strongly (>99%) to plasma proteins, specifically albumin. The long persistence of TCBZ metabolites in the bloodstream (over 120 hours posttreatment) and their high plasma concentrations compared to other BZD compounds, correlate with their high plasma protein binding. These may contribute to its high flukicidal activity, based on a high and prolonged drug exposure of the blood-feeding adult flukes. On the other hand, the metabolic conversion of TCBZSO into TCBZ in the rumen may give rise to a source of the main active metabolite in the liver (Virkel et al., 2006). Thus, the sulforeduction of hydroxy-TCBZSO into hydroxy-TCBZ may be relevant as well, although the mode of action and/or the anthelmintic activity (if any) of these metabolites have not been established. Approximately 45% of the administered dose is eliminated by bile and only a 6.5% is excreted in urine, mainly as TCBZSO and TCBZSO$_2$ (Hennessy et al., 1987).

Flukicidal drugs can reach target sites within *F. hepatica* either by oral ingestion of blood or by transtegumental diffusion. The large absorptive surface area of the fluke's tegument may have a major role in drug diffusion from the surrounding medium. Studies on ex vivo drug diffusion into TCBZ-susceptible *F. hepatica*, demonstrated that TCBZ, TCBZSO, and TCBZSO$_2$ have the capability to penetrate the fluke's tegument (Alvarez et al., 2004; Mottier et al., 2004). Unlike the uptake pattern previously observed for albendazole (Alvarez et al., 2001), the parent TCBZ and its sulfoxide and sulfone metabolites showed a similar ability to penetrate into the trematode parasite. However, the diffusion of the hydroxy-derivatives into the fluke was lower than that observed for TCBZ, TCBZSO, and TCBZSO$_2$. These findings agree with the high correlation observed between lipophilicity and drug concentrations measured within the parasite. Those studies demonstrated that the higher the lipophilicity, the greater the ability of the BZD molecules to cross through the helminth external surfaces. These studies have also shown that the composition and physicochemical characteristics of the incubation medium drastically affect the diffusion of BZD anthelmintics into *F. hepatica*. The time course and pattern of in vivo accumulation of TCBZ and its metabolites in bile, liver tissue, and *F. hepatica* specimens was studied in sheep treated with TCBZ (10 mg/kg) by the intraruminal route (Moreno et al., 2014). Both TCBZSO and TCBZSO$_2$, the only metabolites detected in the bloodstream, were the main metabolic products accumulated within the adult flukes (see Figure 40.15). This finding confirms that oral ingestion is a main route of entry of TCBZ metabolites in vivo. The administered drug (TCBZ) was the main compound accumulated in the liver tissue, whereas low concentrations of this parent drug were recovered from collected parasites. The presence of TCBZ within adult fluke specimens (despite being absent in the bloodstream) may indicate some degree of transtegumental diffusion from bile/liver tissue of treated animals.

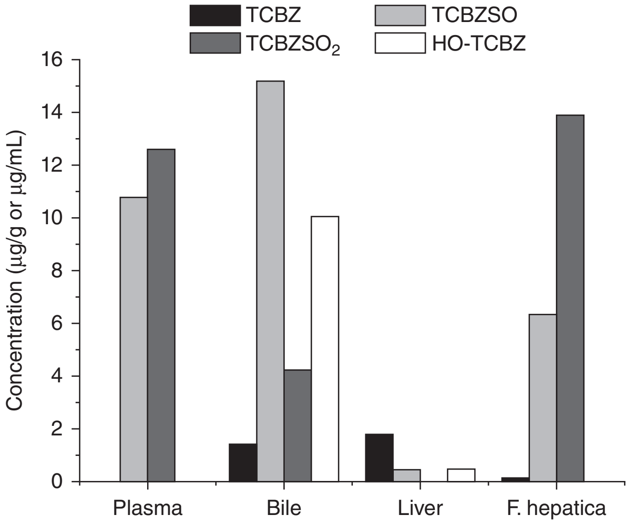


Figure 40.15 Triclabendazole (TCBZ), triclabendazole sulfoxide (TCBZSO), triclabendazole sulfone ($TCBZSO_2$) and hydroxy triclabendazole (HO-TCBZ) concentrations (µg/g or µg/ml) measured in plasma, bile, adult *Fasciola hepatica* and liver tissue at 24 h post-administration of TCBZ (intraruminal at 10 mg/kg) to sheep. See the text for further explanation. Source: Adapted from Moreno et al., 2014. Reproduced with permission of Elsevier.

Since TCBZ is the most widely used flukicide, due to its excellent activity against both the mature and immature stages of *F. hepatica,* the selection of TCBZ-resistant populations is now an emerging problem in several areas of the world (Fairweather, 2005). BZD resistance in *H. contortus* has been associated with the loss of high-affinity binding (Lubega and Prichard, 1991) and an alteration of the β-tubulin isoform pattern. Although the flukicidal activity of TCBZ remains to be fully understood, there are data to support a microtubule-based action of this compound (Stitt and Fairweather, 1994). However, it has been shown that the TCBZ-resistant phenotype in *F. hepatica* is not associated with residue changes in the primary amino acid sequence in β-tubulin (Robinson et al., 2002). This suggests that there may be an alternative mechanism of TCBZ resistance. In fact, the amount of TCBZ recovered from resistant flukes was significantly lower (~50%) than that measured in susceptible parasites. Increased TCBZ oxidative metabolism by the fluke and enhanced drug efflux mediated by ATP-dependent transmembrane transporters may account for the reduced drug accumulation observed in resistant flukes (Alvarez et al., 2005; Mottier et al., 2006), and have been proposed as potential mechanisms of TCBZ resistance in *F. hepatica*. In this regard, *in vitro* studies showed a greater disruption of tegumental, vitellogenic, and spermatogenic cells when TCBZ-resistant specimens of *Fasciola hepatica* were exposed to a combination of TCBZSO and the known P-glycoprotein inhibitor verapamil (Savage et al., 2013, 2014). Conversely, albendazole accumulation was similar in both susceptible and resistant flukes, which correlated with the observed high efficacy of this drug against TCBZ-resistant flukes in the field (Coles and Stafford, 2001) (Figure 40.16)

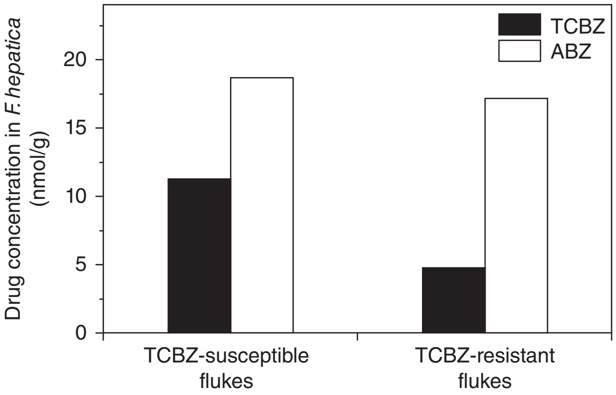


Figure 40.16 Comparative diffusion of triclabendazole (TCBZ) and albendazole (ABZ) into adult specimens of TCBZ-susceptible and TCBZ–resistant *F. hepatica*. See the text for further explanation. Source: Adapted from Mottier et al., 2006. Reproduced with permission of Elsevier.

TCBZ is administered orally to cattle (12 mg/kg), sheep (10 mg/kg), and goats (15 mg/kg), and is indicated for the treatment and control of all stages of TCBZ susceptible *F. hepatica* from 2-day-old early immature to mature fluke, and so is indicated against both acute and chronic fasciolosis. Combined formulations containing TCBZ and different antinematodal drugs (levamisole, abamectin, ivermectin) are available in different countries. The pharmacokinetic properties of both TCBZ and ivermectin coadministered in sheep were evaluated (Lifschitz et al., 2009). Ivermectin elimination was delayed and its plasma availability was threefold higher when given in combination with TCBZ. Similarly, plasma concentrations of TCBZ and its metabolites were influenced by its coadministration with ivermectin. Higher peak plasma concentrations of TCBZ metabolites were observed after the coadministration of TCBZ and ivermectin compared to those obtained following TCBZ alone treatment. It was suggested that TCBZ may modulate ivermectin biliary and/or intestinal P-glycoprotein-mediated elimination. The same rationale was applied for the observed modifications on the systemic exposure of TCBZ and its active metabolite TCBZSO because both molecules were shown to interact with P-glycoprotein (Dupuy et al., 2010).

TCBZ can be administered at any stage of pregnancy. A withdrawal time of 56 days (meat) has been established for TCBZ in sheep and cattle. TCBZ is not indicated to be used in lactating animals producing milk for human consumption, or in pregnant animals that are intended to produce milk for human consumption within 2 months

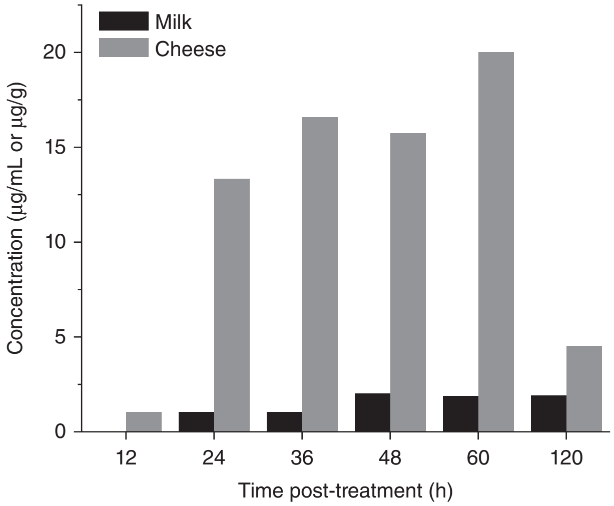


Figure 40.17 Comparison of the total residue levels (sum of triclabendazole sulfoxide and triclabendazole sulfone concentrations) measured in pooled milk and cheese elaborated with milk collected from treated cows at each time interval. Source: Adapted from Imperiale et al., 2011. Reproduced with permission of Taylor & Francis. Food Additives and Contaminants, 28(4):438-445.

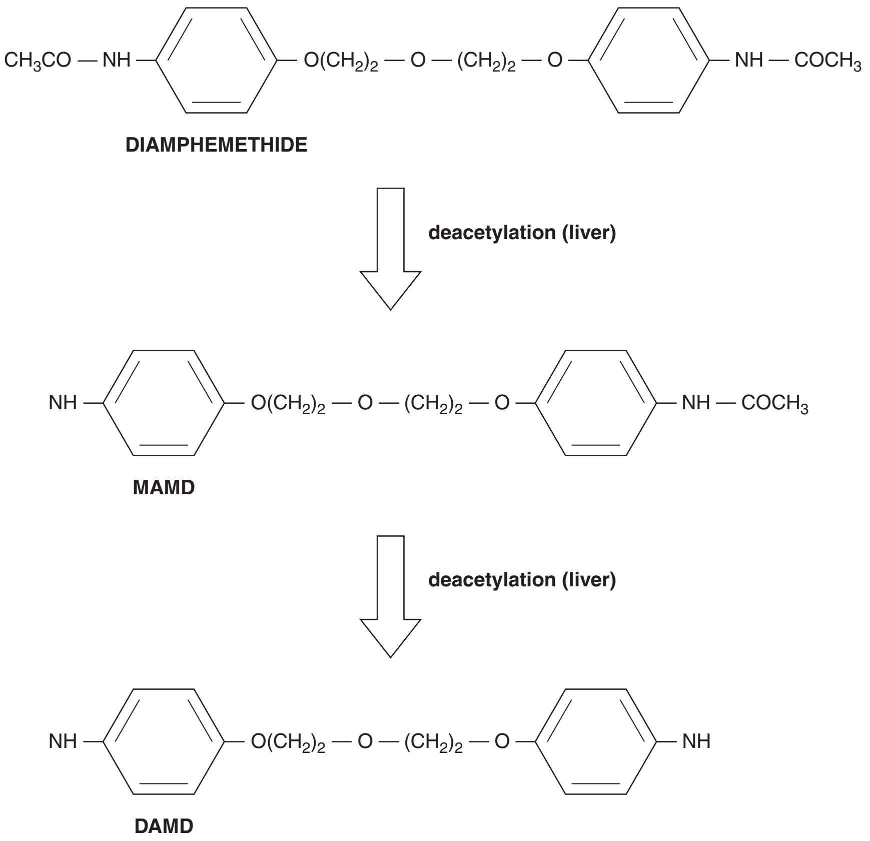
of expected parturition. Both TCBZSO and $TCBZSO_2$ were detected in milk from lactating dairy cattle (up to 36 and 144 hours, respectively) when TCBZ was administered at 12 mg/kg by the oral route (Imperiale et al., 2011). The total average residual concentration in fresh cheese was 13-fold higher than that obtained in milk used for its elaboration (see Figure 40.17). These results reaffirm why TCBZ is banned for use in lactating animals.

Phenoxyalkanes

Diamphenethide

This flukicidal molecule is a phenoxyalkane (*N,N′*-[oxybis(2,1-ethanediyloxy-4,1-phenylene)] bisacetamide) (Figure 40.18). Diamphenetide is exceptionally effective against early immature flukes from 1 day to 6 weeks of age. Consequently, it is used for treatment of acute fasciolosis resulting from immature forms of *F. hepatica* migrating through the liver parenchyma of sheep. A dosage of 100 mg/kg is almost 100% effective against flukes from 1 to 63 days of age. However, its activity progressively decreases as the flukes develop to maturity. This is in direct contrast to other currently used fasciolicides, which tend to be less active against younger flukes. This phenomenon is a drawback for the clinical use of diamphenethide but the drug may be useful in prophylactic programs against liver fluke disease in sheep. A chemoprophylactic effect has been achieved as a result of strategic treatments with diamphenetide in both sheep and cattle (Enzie et al., 1980). At a dosage of 10 mg/kg of body weight (10% of the recommended dose), the drug

Figure 40.18 Diamphenetide: chemical structure and metabolic fate. Liver deacetylation of the parent drug renders into two active diamino metabolites: monoaminodiamphenethide (MAMD) and diaminodiamphenethide (DAMD).



resulted 87% effective in preventing establishment of *F. hepatica* infections in sheep that were given the drug daily for 14 days, and was 96% effective in sheep that were given the drug for 21 days (Enzie et al., 1980). It has also been proposed the use of a controlled-release device of diamphenethide for the prophylaxis of fasciolosis in ruminants (Rew and Knight, 1980).

The efficacy of diamphenethide depends upon deacetylation of the drug by liver enzymes (deacetylases) to an amine metabolite. Diamphenethide is not active in vitro, but its deacetylated (amine) metabolite (DAMD) has flukicidal activity against liver flukes. The exact mechanism of action of DAMD remains not well established as yet. Although it was shown that DAMD caused severe morphological damage to *F. hepatica* in vitro, energy metabolism is not the prime target for this metabolite (Fairweather and Boray, 1999). DAMD-induced disruption of the tegument and osmoregulatory system of the fluke was thoroughly described using transmission electron microscopy (Anderson and Fairweather, 1995). Morphological changes include swelling of the in-foldings of the basal plasma membrane leading to flooding of the syncytium and eventual sloughing of the tegument. Further information on the mode of action of DAMD can be obtained from Fairweather and Boray (1999).

Diamphenethide is administered as an oral suspension at 100 mg/kg in sheep and goats. Diamphenethide is absorbed into the blood after oral treatment and readily metabolized in the liver. High concentrations of two active deacetylated metabolites, monoamino- (MAMD) and diamino-diamphenethide (DAMD) are produced in liver parenchyma, where immature flukes are found until at least 7 weeks after infection. Metabolites reach high concentrations in the liver parenchyma but are thought to be rapidly biotransformed and eliminated into the bile. Only small amounts of MAMD and DAMD may escape into the bloodstream but become diluted. This is why the quantity of active metabolites reaching mature blood-sucking flukes by both oral ingestion or tegumental diffusion is small. Therefore, efficacy against adult stages located in the bile ducts is reduced.

Safety of diamphenetide for the host can be explained on the basis of destruction of the metabolites by the liver and dilution in blood so that only small quantities reach other tissues. Its greatest concentrations are measured in the liver and gallbladder content (at 3 days posttreatment). It should be noted that adult flukes reside in intimate association with the biliary system. At 7 days after dosing, concentrations of diamphenethide in these sites are reduced approximately 10-fold, to a range of only 0.1–0.5 ppm, while concentrations in the musculature are approximately 0.02 ppm. In the United Kingdom, animals intended for human consumption are permitted to be slaughtered 7 days after treatment. The usual oral dose (100 mg/kg) in sheep is apparently safe. A single oral dose four times the therapeutic dosage (400 mg/kg) produces no toxic signs. At higher dosages, toxic effects include temporary impairment of vision and loss of wool. Pastured sheep are less susceptible to toxic effects than housed sheep. There appear to be no significant contraindications for use of diamphenethide. Pregnant ewes dosed with 200 mg/kg once weekly on 2, 3, or 4 consecutive occasions during the 21-week gestation period exhibited no adverse effects on fertility or teratogenic effects in their offspring.

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41

Macrocyclic Lactones: Endectocide Compounds

Carlos E. Lanusse, Fernanda A. Imperiale, and Adrian L. Lifschitz

General Pharmacology: Avermectins and Milbemycins

The integration of available information on the host–parasite–environment relationship with a more complete understanding of the pharmacological properties of existing antiparasitic drugs should result in more efficient parasite control in domestic animals. The time of parasite exposure to active drug concentrations determines the efficacy and/or persistence of activity for the most of the anthelmintic drugs used in veterinary medicine. The characterization of drug concentration profiles in tissues of parasite location and within target parasites provides a basis for understanding the differences in therapeutic and preventive efficacies observed for the different anthelmintic chemical families.

The avermectins and milbemycins are closely related 16-membered macrocyclic lactones (MLs), produced through fermentation by soil dwelling actinomycetes (*Streptomyces*). It was the unique combination killing of endo- and ectoparasites that gave rise to the embracing name *endectocide* by which the MLs are recognized (Shoop et al., 1995a). Information on the antiparasitic properties of the different ML compounds in all animal species has been thoroughly reviewed elsewhere (Campbell, 1985; Shoop et al., 1995a; McKellar and Benchaoui, 1996). Specific literature devoted to the MLs (Vercruysse and Rew, 2002; Gonzalez-Canga, 2012), is recommended for readers interested on any further detailed issue within the topic. Enormous progress on understanding the relationship among disposition kinetics, tissue distribution, and the patterns of antiparasitic persistence for the ML molecules has been achieved, particularly in ruminant species. The purpose of this chapter is to provide an updated and integrated overview of the main basic pharmacological features of the ML compounds, which account for their outstanding long-persistent broad-spectrum antiparasitic activity. The MLs are now considered the most widely used broad-spectrum antiparasitic drugs in veterinary medicine. Chapter 43 describe MLs use as ectoparasiticides.

Avermectins are produced as a mixture of different components from fermentation of *Streptomyces avermitilis*. These natural products are denoted as A (those containing a methoxy group at the 5-position) and B (with a hydroxy group at the 5-position). The highest anthelmintic potency is held by the B1 homologs. The avermectin family includes a series of natural and semisynthetic molecules, such as *abamectin*, *ivermectin*, *doramectin*, *eprinomectin*, and *selamectin*. Abamectin is the naturally occurring avermectin approved for animal use and the starting material for the production of ivermectin. In fact, ivermectin, the first marketed endectocide molecule, is a semisynthetic derivative of the avermectin family. Ivermectin is the generic name given to a mixture of two chemically modified avermectins containing at least 80% of 22-23-dihydroavermectin B1a and less than 20% of 22-23-dihydroavermectin B1b (Fisher and Mrozik, 1989). Doramectin was obtained by mutational biosynthesis in which the precursor (cyclohexanecarboxylic acid) was fed to a mutant strain of *Streptomyces avermitilis* (Goudie et al., 1993). Eprinomectin is a semisynthetic avermectin compound, developed for topical use in cattle (Shoop et al., 1996). Selamectin is a semisynthetic monosaccaride oxime derivative of doramectin more recently introduced into the market for parasite control in dogs and cats (Bishop et al., 2000). The main chemical differences among the avermectin-type compounds are shown in Figure 41.1. It has been shown that minor chemical changes may have a profound impact on the activity of the avermectin analogs. For instance, substitution of the C-5 hydroxy with an oxo-substituent on ivermectin or doramectin monosaccharides can drastically reduce activity against *Haemonchus contortus* larvae.

Nemadectin, *moxidectin*, and *milbemycin oxime* belong to the *milbemycin* family. Milbemycin oxime consists of two oxime derivatives (A_4 and A_3 components) of the 5-didehydromilbemycin in an 80 : 20 ratio. Moxidectin is a milbemycin compound produced by a combination of fermentation and chemical synthesis. Moxidectin is obtained by chemical modification of

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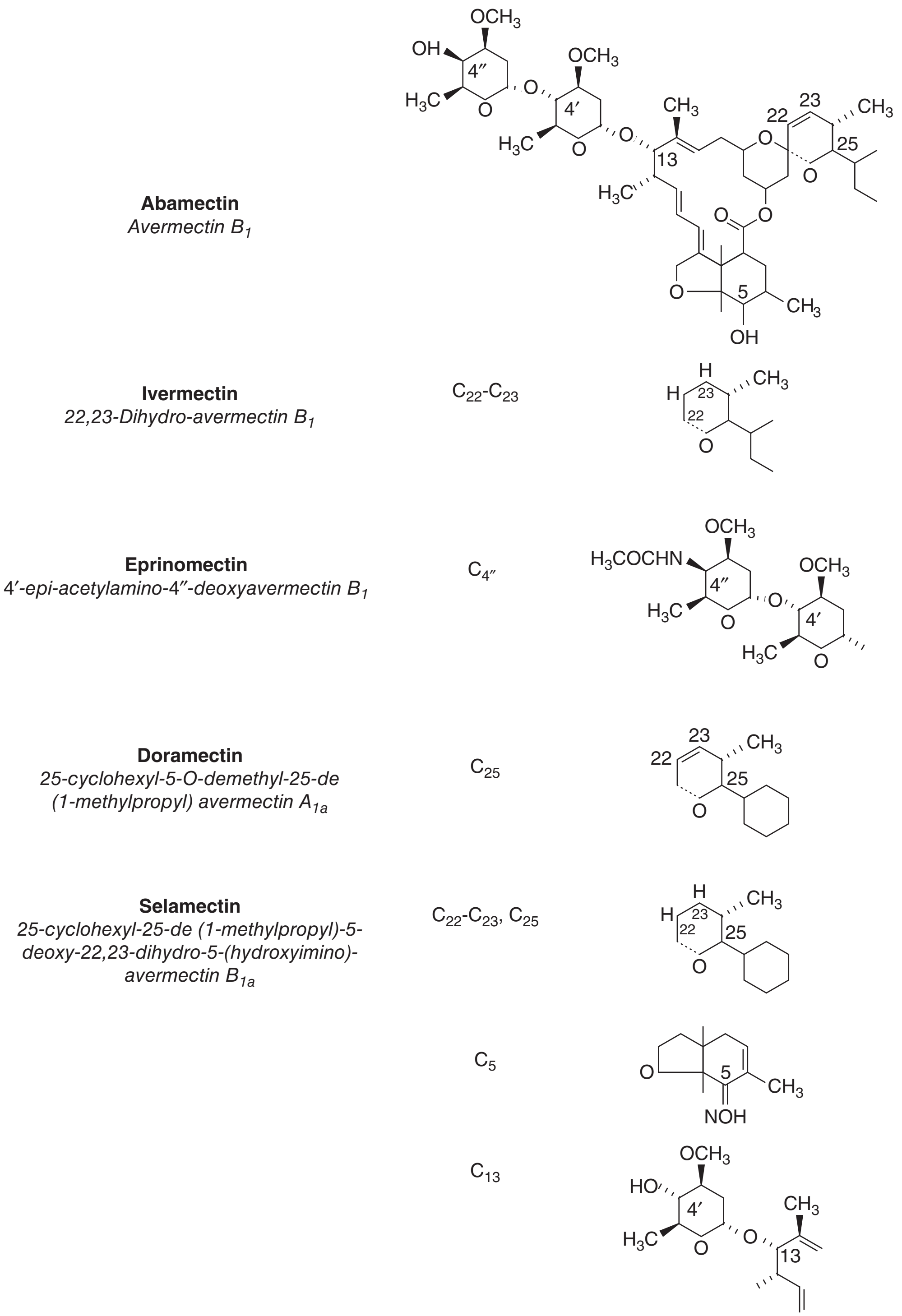
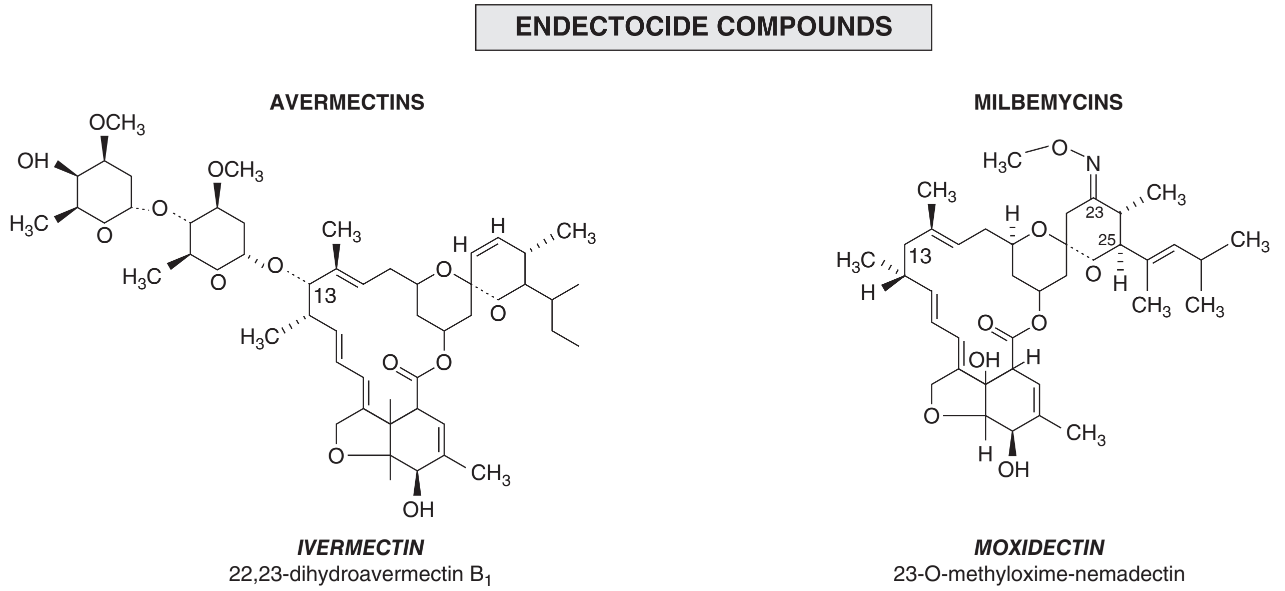

Abamectin
Avermectin B1
Ivermectin
22,23-Dihydro-avermectin B1
C22-C23
Eprinomectin
4′-epi-acetylamino-4″-deoxyavermectin B1
C4″
Doramectin
25-cyclohexyl-5-O-demethyl-25-de (1-methylpropyl) avermectin A1a
C25
Selamectin
25-cyclohexyl-25-de (1-methylpropyl)-5-deoxy-22,23-dihydro-5-(hydroxyimino)-avermectin B1a
C22-C23, C25
C5
C13
OCH3
OH
4″
H3C
O
4′
CH3
22
23
25
13
5
OH
H
H3COCHN
NOH
HO

Figure 41.1 Chemical structures of the main avermectin compounds used in veterinary medicine. The main chemical differences between abamectin (the natural fermentation product) and other avermectin-type molecules are shown. The commercially available preparations of abamectin and ivermectin contain at least 80% of the avermectin B_{1a} (the a-component has a secondary butyl side chain at the 25-position) and no more than 20% of avermectin B_{1b} (the b-component has an isopropyl substituent at the 25-position). Eprinomectin contains at least 90% of the B_{1a} and no more than 10% of the B_{1b} component.



- **16-membered macrocyclic lactones**
- **High lipophilicity**
- **Common mode action**
- **Potent broad spectrum**
- **Persistent ecto-endoparasitic activity**

Figure 41.2 Comparison between avermectin (ivermectin) and milbemycin (moxidectin) molecular structures. The main pharmacological features shared by compounds in both families are listed. The main chemical differences between ivermectin and moxidectin are at the 13-, 23-, and 25-positions (see text for further explanation).

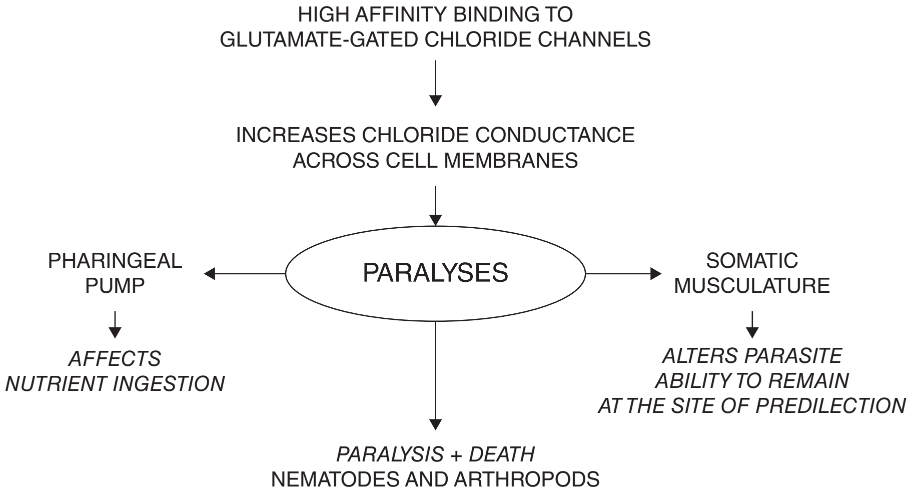
nemadectin, the natural compound produced when *Streptomyces cyaneogriseus* is grown under controlled culture conditions (Takiguchi et al., 1980). The molecular structures of the two endectocide families are superimposable (Shoop et al., 1995a); however, milbemycins do not have the C_{13} disaccharide substituent in the macrolide ring (Figure 41.2). Additionally, moxidectin differs from ivermectin at the 23-position, where it possess a metoxime moiety. Moxidectin also has a differential substituted olefinic side chain at the 25-position. In spite of these chemical differences, both families share some structural and physicochemical properties, and their broad-spectrum antiparasitic activity against nematodes and arthropods at extremely low dosage rates (Figure 41.2).

Various compounds from both families are used in livestock, companion animals, wildlife species, and humans (ivermectin). Abamectin, ivermectin, doramectin, and moxidectin, currently marketed as injectable, pour-on (cattle), and oral (sheep, goats) formulations, are the most commonly used MLs worldwide to control endo- and ectoparasites in livestock. Exceptional potency, high lipophilicity, and the prolonged persistence of their potent broad-spectrum activity are distinctive features among antiparasitic drugs.

Mechanism of Action: Ecto–Endoparasiticadal Activity

The broad-spectrum ecto–endoparasitic activity of the ML from both families includes selective toxic effects on insects, acarines, and nematodes. However, they do not possess efficacy against cestode and trematode parasites. The MLs induce reduction in motor activity and paralysis in both arthropods and nematodes. The paralytic effects are mediated through GABA and/or glutamate-gated chloride channels (GluCl), collectively known as ligand-gated chloride channels. MLs may act on both receptors and a putative GABA-receptor subunit has been localized along the ventral nerve cord and several neurons in the head of *H. contortus* (Blackhall et al., 2003). However, considerably higher ML concentrations are required for the GABA-mediated effects than for those on the GluCl channels. In fact, a high affinity binding to GluCl channels, producing a slow and irreversible increase in

Figure 41.3 Schematic representation of the proposed mode of action for the macrocyclic lactone endectocides.


HIGH AFFINITY BINDING TO GLUTAMATE-GATED CHLORIDE CHANNELS
INCREASES CHLORIDE CONDUCTANCE ACROSS CELL MEMBRANES
PARALYSES
PHARINGEAL PUMP
AFFECTS NUTRIENT INGESTION
SOMATIC MUSCULATURE
ALTERS PARASITE ABILITY TO REMAIN AT THE SITE OF PREDILECTION
PARALYSIS + DEATH NEMATODES AND ARTHROPODS

chloride membrane conductance (Forrester et al., 2003), hyperpolarization, and flaccid paralysis of the invertebrate somatic muscles is now proposed as a main mode of action (Martin et al., 2002; Geary, 2005). The ML endectocides cause paralysis and death of both arthropod and nematode parasites due to their paralytic effects on the pharyngeal pump (Geary et al., 1999), which affects nutrients ingestion, and on the parasite somatic musculature limiting its ability to remain at the site of predilection in the host. In addition, inhibitory effects of the MLs on the female reproductive system may explain the observed reductions in parasite egg production (Fellowes et al., 2000). In *H. contortus*, the pharynx appears to be the most sensitive of the affected muscles (Sheriff et al., 2005). A schematic representation of the proposed main mode of action for the ML endectocides is shown in Figure 41.3.

The GluCl channels are members of the ligand-gated ion channel superfamily uniquely found in invertebrates. Some channel subunits have now been cloned from different nematode parasites, including the free-living nematode *Caenorhabditis elegans*, *H. contortus,* and *Cooperia* spp. (Forrester et al., 2003; Njue and Prichard, 2004), which has facilitated enormous progress in the understanding of the mode of action and mechanisms of resistance of the MLs. A good correlation between the presence of GluCl on neurons and muscle cells and the electrophysiological effects induced by MLs has been observed (Martin et al., 2002). Although several GluCl channel subunits have been identified, the sensitivity to these drugs seems to be associated with the alfa-subunit of the receptor (Arena et al., 1992; Cully et al., 1994; Njue and Prichard, 2004), which is encoded by different genes indicating that nematode resistance to these compounds may be polygenic.

The different ML compounds exhibit a similar, but not identical, spectrum of activity against arthropod and nematode parasites and also show differences on the persistence of their antiparasitic activity. Even though compounds in both the avermectin and milbemycin families appear to have the same mode of action, there is now evidence of some subtle pharmacodynamic differences between families, which seems to account for a higher pharmacological potency of moxidectin compared to ivermectin, particularly evident against resistant nematode parasites. These pharmacodynamic differences have been observed at the GluCl channel where moxidectin is 2.5-fold more potent than ivermectin in terms of the required EC_{50} to activate this receptor expressed in *Xenopus* oocytes (reviewed by Prichard et al., 2003). More recently, Hibbs and Gouaux (2011) proposed a model for the ivermectin binding interactions with amino acids at the GluCl channel of *C. elegans*. Considering the structural differences between moxidectin and ivermectin, it is likely that interaction of moxidectin with the target parasite receptor may differ from that of the avermectins (Prichard et al., 2012).

In contrast to mammals, nematodes have multiple genes coding for transporter proteins, which may be essential to protect them from a broad spectrum of toxins (Prichard and Roulet, 2007). The ATP-binding cassette (ABC) transporters are a family that includes different proteins such as P-glycoprotein (P-GP), multidrug resistance proteins (MRPs), and breast cancer resistant protein (BCRP). P-GP acts as an efflux pump for ML compounds, lowering the local drug concentration achieved at the GluCl receptor site of action. Whereas 14 P-GP encoding genes have been identified in the genome of *C. elegans*, sequences of nine P-GPs have been described in *H. contortus* (Williamson and Wolstenholme, 2012). Different P-GPs have been described in other nematodes, such as *O. volvulus* and cyathostomins. As it was demonstrated with mammalian transporters (see Chapter 50 for further discussion), it seems that they play a similar role in modulating MLs uptake and excretion in nematodes, which may modulate the MLs concentrations reaching the target site. Many studies have shown the role of transporter proteins as a potential mechanism of drug resistance in different helminth species, as it was reviewed in detail by Lespine et al. (2011). In vitro and ex vivo studies have provided clear evidence that moxidectin has a lower affinity for P-GP compared to avermectin-type compounds (Lespine et al., 2007; Ballent et al., 2014). This enhanced ivermectin efflux may account for lower active concentrations achieved at the receptor site in comparison to moxidectin. Data demonstrate minor pharmacodynamic differences between ivermectin and moxidectin, which could explain differences in spectrum and potency, and the advantageous pattern of moxidectin efficacy against ivermectin-resistant nematodes of sheep, goats, and cattle.

Pharmacokinetics

Exchange Between Bloodstream and Target Tissues

The clinical efficacy of the MLs is closely related to their pharmacokinetic behavior, that is the time of parasite exposure to active drug concentrations, which is required to obtain optimal and persistent antiparasitic activity. Their antiparasitic spectrum and efficacy pattern are similar; however, each compound has its own dosage limiting species. Differences in physicochemical properties may account for differences in formulation flexibility, kinetic behavior, and in the potency/persistence of their endectocide activity. Thus, even slight modifications to the disposition kinetics or to the pattern of plasma/tissue exchange may notably affect the persistence of their antiparasitic effect.

An enormous effort has been devoted to characterization of the kinetics of the different MLs in different species. The work on the comparative plasma (Lanusse et al., 1997; Toutain et al., 1997) and target tissues (Lifschitz et al., 1999a, 2000; Sallovitz et al., 2003) disposition kinetics of ivermectin, doramectin, and moxidectin in cattle, the disposition of doramectin (Hennessy et al., 2000; Carceles et al., 2001) and moxidectin (Alvinerie et al., 1998a) in sheep and goats, including evidences on their metabolic stability in the digestive tract (Lifschitz et al., 2005) and involvement of lymphatic transport on intestinal absorption (Lespine et al., 2006a), have contributed greatly to the understanding of the overall ML pharmacokinetic properties. Additionally, the characterization of the plasma/milk profiles of eprinomectin in cattle and goats (Shoop et al., 1996; Alvinerie et al., 1999a), the studies on the comparative milk excretion patterns of different ML in dairy sheep, and their impact on drug residue profiles in milk-derived products (Imperiale et al., 2004a), have further contributed to the knowledge on ML kinetic behavior. The main pharmacokinetic features accounting for the persistent broad-spectrum antiparasitic activity of endectocides in ruminants are summarized in Figure 41.4.

Plasma concentration profiles may help to predict the persistence of antiparasitic activity, particularly after parenteral administration. However, the measurement of drug concentration profiles at the site of parasite location and thus antiparasitic activity (the so-called biophase in the pharmacodynamic models of Chapter 4), permits a more direct interpretation and provides a basis for understanding the differences in therapeutic and preventive efficacies observed for MLs. The characterization of drug concentrations at the sites of parasite infection for moxidectin, ivermectin, and doramectin in cattle, and the characterization of the kinetic disposition of doramectin

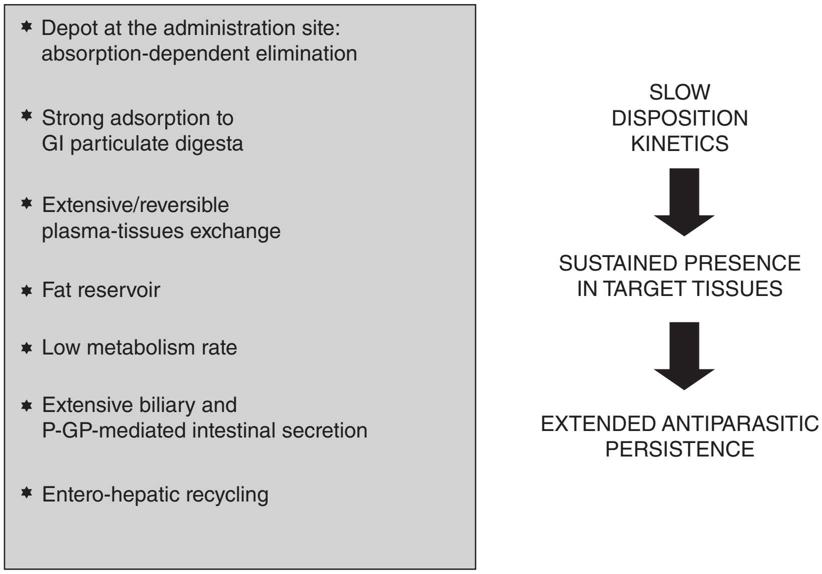

* Depot at the administration site: absorption-dependent elimination
* Strong adsorption to GI particulate digesta
* Extensive/reversible plasma-tissues exchange
* Fat reservoir
* Low metabolism rate
* Extensive biliary and P-GP-mediated intestinal secretion
* Entero-hepatic recycling
SLOW DISPOSITION KINETICS
SUSTAINED PRESENCE IN TARGET TISSUES
EXTENDED ANTIPARASITIC PERSISTENCE

Figure 41.4 Summary of the main pharmacokinetic features accounting for the extended broad-spectrum antiparasitic persistence of the macrocyclic lactone compounds in ruminants. GI, gastrointestinal tract; P-GP, P-glycoprotein. Source: Adapted from Lifschitz et al., 2000.

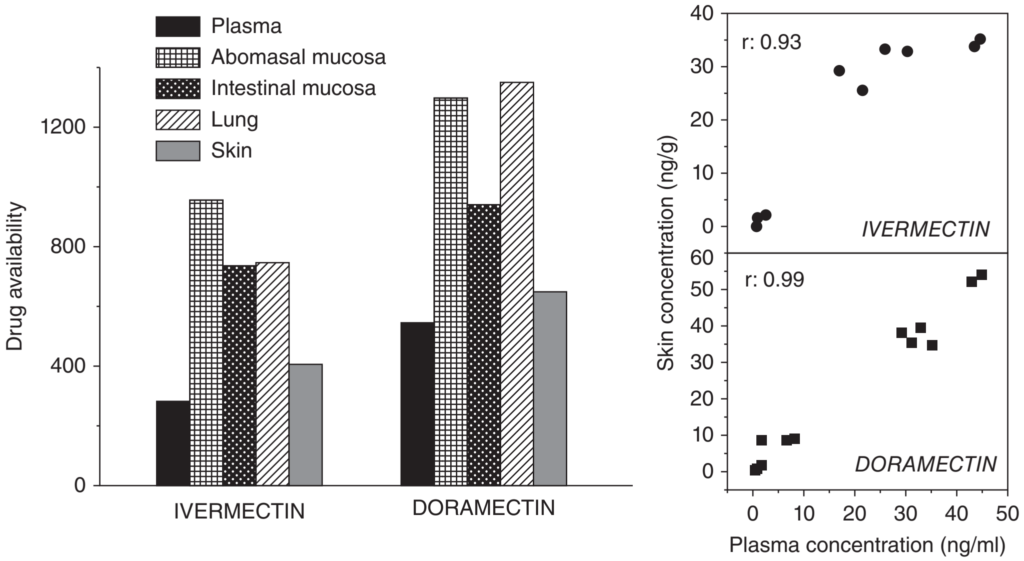


Figure 41.5 Pattern of ivermectin and doramectin distribution to parasite location tissues in subcutaneously treated calves (0.2 mg/kg). Drug availability is expressed as area under the concentration vs. time curve (AUC) in ng.day/ml (plasma) and ng.day/g (target tissues). The correlations between drug concentrations measured in plasma and in a target tissue (skin) are shown in the right panel. r, correlation coefficient. Source: Data from Lifschitz et al., 2000.

in fluid and particulate digesta throughout the gastrointestinal tract in sheep, represent a considerable contribution to the understanding of the comparative persistence of activity of these compounds in ruminants. The highly lipophilic MLs are extensively distributed from the bloodstream to different tissues. Their extensive tissue distribution agrees with the high availability of these drugs in different parasite location tissues such as the gastrointestinal mucosal tissues, lungs, and skin in cattle (Figure 41.5), where concentrations markedly greater than those observed in plasma were measured during 50–60 days posttreatment. A strong correlation between plasma and tissue concentration profiles has been observed after the subcutaneous administration of ML compounds. The high correlation coefficients obtained showed the degree of association between ML concentrations in the bloodstream and those measured in tissues of parasites location. Despite the high correlation observed between concentrations in plasma and in tissues/fluids, ivermectin, doramectin, and moxidectin concentrations were greater in all target tissues compared to plasma.

MLs are extremely effective against adult and larval stages of most gastrointestinal nematodes and *Dictyocaulus viviparus* in the lung in cattle, exerting a prolonged protective effect. The achievement of high drug availability in lung tissue may explain their excellent activity against lungworms. The pulmonary tract was the target tissue in which the highest doramectin concentrations were recovered in parenterally treated cattle (Lifschitz et al., 2000). The persistence of the broad-spectrum anthelmintic activity of the MLs against adult and immature gastrointestinal parasites is facilitated by their advantageous pattern of distribution and prolonged residence in the digestive mucosal tissue. Differences in the plasma pharmacokinetic profiles between doramectin and ivermectin have been reported in cattle. Following intravenous administration of an aqueous micelle formulation to determine the intrinsic kinetic features of the compounds, higher plasma concentrations and delayed elimination of doramectin compared to ivermectin were reported (Goudie et al., 1993). After subcutaneous administration of the commercially available formulations to cattle, higher plasma concentrations and extended plasma residence were observed for doramectin compared to ivermectin. The kinetic variables obtained for ivermectin and doramectin in tissues of parasite location are consistent with the described plasma kinetics data. Enhanced availability and prolonged time of residence in target tissues were observed for doramectin compared to ivermectin. The oil based formulation of doramectin, potentially coupled to a slowed metabolic rate based on the presence of the cyclohexyl group at C_{25} (Goudie et al., 1993), may contribute to the higher plasma and target tissues drug availability of doramectin compared to ivermectin.

Moxidectin is the most lipophilic endectocide agent; its high lipophilicity results in a wide tissue distribution (volume of distribution = 13.6 l/kg) and long residence in plasma, which is clearly reflected in the tissue pharmacokinetic results obtained in cattle. Distribution of the drug into adipose tissue accounts for the larger distribution for this compound compared to other antiparasitic drugs. The concentration of moxidectin in fat after

28 days of treatment in cattle has been shown to be 90-fold higher than that detected in plasma (Zulalian et al., 1994). The large tissue distribution of moxidectin in cattle agrees with the high availability of moxidectin in the gastrointestinal mucosal tissues, lungs, and skin, with concentrations ranging between 1 and 2 ng/g at 28 days posttreatment, and with the extended detection of moxidectin concentrations >0.1 ng/g up to 58 days posttreatment in those tissues. The high C_{max} values and total drug availability obtained in tissues where target parasites are located is in agreement with the extensive tissue distribution of moxidectin, and thus may be relevant in terms of antiparasitic activity against internal and external parasites. Long residence times for moxidectin (between 6.8 and 11.3 days) were obtained in the different sites of parasite location, which agrees with the extended residence of moxidectin reported in fat and in the bloodstream (Alvinerie and Galtier, 1997; Lanusse et al., 1997). Moxidectin depletion half-lives in target tissues ranged from 7.73 days (skin) to 11.8 days (intestinal mucosa) (Lifschitz et al., 1999a), which were significantly longer than those determined for ivermectin and doramectin in the same tissues. The deposit of moxidectin in adipose tissue may act as a drug reservoir that contributes to the long persistence of this molecule in the bloodstream and in different tissues of parasite location. The estimated plasma elimination half-lives for different ML compounds in cattle are shown in Figure 41.6. Drug elimination half-life values for the main host tissues where target endo- and ectoparasites are located, are also shown.

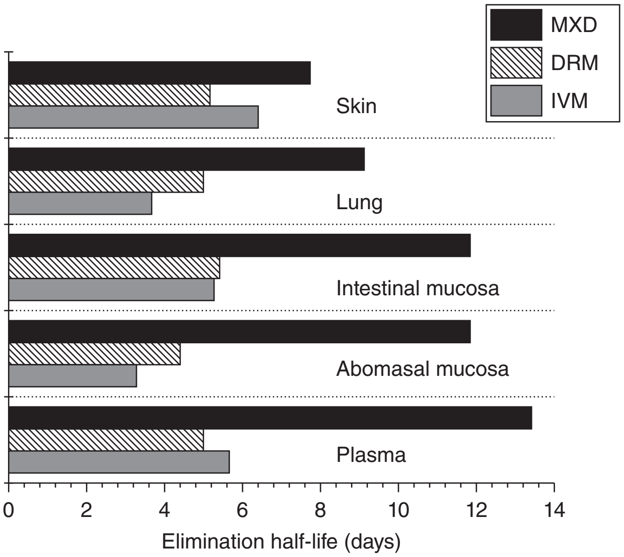


Figure 41.6 Comparative mean elimination half-lives for ivermectin, doramectin, and moxidectin in plasma and tissues of parasite location after their subcutaneous administration to cattle (0.2 mg/kg). Source: Data from Lifschitz et al., 1999a, 2000.

A considerable excretion of ivermectin, moxidectin, and doramectin occurs via mammary glands, which invalidate their use in dairy animals.

However, topical formulations of moxidectin are currently approved for use in dairy cattle in some countries without a milk withdrawal time. Although licking behavior may drastically enhance milk residues of topically administered moxdectin, the residual concentrations reported did not exceed the permitted residual concentrations at any time posttreatment (Imperiale et al., 2009). Topical application is fully discussed in Chapter 43.

In an effort to identify an ML molecule that could be used in dairy cattle, an avermectin-derivative, named eprinomectin, has been developed for topical administration to cattle. While the milk/plasma partitioning for eprinomectin in topically treated cattle falls between 0.1 and 0.2 (Shoop et al., 1996; Alvinerie et al., 1999a), the milk/plasma ratio for ivermectin is between 1 (goats and cattle) (Alvinerie et al., 1993; Alvinerie et al., 1996) and 2 (sheep) (Imperiale et al., 2004b). The high lipid content of the milk may explain the higher rate of ivermectin transfer into sheep's milk. The partitioning of moxidectin into sheep's milk is higher than that of ivermectin with milk/plasma concentration ratios between 10.0 and 18.0 (Imperiale et al., 2004a, 2004b). This is consistent with the higher lipophilicity of moxidectin compared with ivermectin.

Eprinomectin, in addition to its antiparasitic potency and broad spectrum, has a substantially reduced distribution to milk, compared to other MLs, which is probably based in some minor changes introduced to its chemical structure. In fact, changes to the milk partitioning coefficients in lactating dairy animals were achieved by manipulation of the avermectin molecule. The C4″-epi-amino analogues unsaturated at $C_{22\text{-}23}$ showing the lowest distribution into milk was eprinomectin, 4″-epi-acetylamino-4″-deoxyavermectin B_1 (see Figure 41.1), and was the best performing compound in terms of broad-spectrum antiparasitic activity.

Hepatic Metabolism: Bile and Intestinal Excretion

Although some metabolic products have been recovered in plasma after administration of different MLs to cattle, these molecules are minimally metabolized in sheep and cattle, with bile and feces the major routes of excretion for unchanged parent drugs. The major liver metabolite of ivermectin in cattle is a 24-hydroxymethyl-dihydroavermectin B_{1a} derivative (Chiu et al., 1987), identical to that produced by steer and rat microsomes in vitro (Miwa et al., 1982). Similarly, a doramectin metabolite, accounting for 5.75% of the total recovered parent drug, was detected in plasma between 8 hours and 45 days postadministration of doramectin. The metabolism of moxidectin has been shown to produce the C_{29-30} and the C_{14} monohydroxymetyl derivatives as the main metabolic products. These and other hydroxylated metabolites have been recovered from different tissues of moxidectin-treated cattle (Zulalian et al., 1994). More extensive biotransformation of moxidectin, compared to ivermectin and doramectin, would agree with the higher ratio of metabolites/parent drug recovered in plasma of moxidectin-treated animals. Interestingly, while 90% of the fat residue at 21 days posttreatment is moxidectin parent drug, ivermectin parent compound accounts only for an 18% of the total fat residue at the same time posttreatment in cattle.

The avermectins are excreted in high concentration in bile and feces of treated cattle. Following an intraruminally administered dose of doramectin in sheep, 25% is secreted in bile, with 20% of biliary metabolites enterohepatically recycled (Hennessy et al., 2000). Consistently, high concentrations of unchanged moxidectin are excreted by bile and feces (Zulalian et al., 1994), with concentrations greater than 2 ng/ml (ng/g) recovered both in bile and feces up to 48 days posttreatment in cattle (Lifschitz et al., 1999a). As pointed out by Hennessy (2000), the extent to which the biliary-secreted drug and metabolites are presented to the gut lumen, reabsorbed as free compound, and subsequently participate in enterohepatic cycling is a major contributor to parasite exposure. See Chapter 2 for an introduction to this process.

The MLs abamectin, ivermectin, and moxidectin (Pouliot et al., 1997; Dupuy et al., 2001), among many other substances, have been shown to be substrates of the P-GP transport protein. In the mammalian host, P-GP participates in the mechanism of active biliary and intestinal secretion of different molecules from the bloodstream to the gastrointestinal tract. The transepithelial intestinal secretion plays a major role in the elimination of ivermectin in the rat (Laffont et al., 2002). Ivermectin clearance from the small intestine accounted for 27% of the total drug clearance, while bile secretion accounted for only 5% of the total clearance in the rat.

Pharmacokinetic–Pharmacodynamic Relationship

The MLs induce a paralytic effect on the pharynx and somatic musculature of nematodes. Different in vitro assays have been used to evaluate ML effects on nematodes. The migration inhibition assay (LMIA) can be used to detect effects on somatic muscle and related neuromuscular sites. On the other hand, the larval development assay (LDA) is not specific for a particular organ and may involve pharyngeal and/or somatic muscle (Demeler et al., 2013a). A different potency among MLs has been shown with the LDA. Abamectin and moxidectin were the most potent compounds against susceptible and resistant *H. contortus* and *H. placei* strains (Kotze et al., 2014). Ivermectin concentrations of 1 nM paralyze the pharynx of *H. contortus* (Geary et al., 1993) and inhibit larval development of trichotrongyloid nematodes (Gill and Lacey, 1998), while much higher concentrations of ivermectin are needed to obtain an inhibitory effect of larval motility (Gill et al., 1991). Considering that 1 nM of ivermectin equals 0.87 ng/g, it may be possible to establish a correlation between the concentrations used in these in vitro assays with those obtained in target tissues after in vivo treatment. Therefore, it could be assumed that the period of time during which drug concentrations are above 1 ng/g in target tissues would be indicative of the period during which a paralytic effect on nematode pharyngeal pump occurs. The ML concentrations required at the site of parasite location to inhibit parasite establishment or development have not been determined. However**,** it has been theoretically assumed that the period of time during which drug concentrations are above 1 ng/g (the ivermectin concentration equivalent to that required to paralyze the pharynx of *H. contortus* in different in vitro pharmacodynamic assays) in target tissues, would be indicative of the period during which the in vivo anthelmintic activity may persist. While ivermectin concentrations were >1 ng/g in gastrointestinal mucosal tissue and lung tissue for 18 days posttreatment, doramectin and moxidectin profiles remained above that level for 38 days postadministration (Lifschitz et al., 2000). The comparison between these time periods and those obtained in different persistence efficacy trials is interesting. The increases of fecal eggs counts after ivermectin and doramectin administration to cattle indicate that reinfection begins 2–3 weeks and 3–4 weeks after each treatment, respectively (Ranjan et al., 1997). The characterization of drug concentrations in target tissues is useful to estimate the period of time posttreatment in which the endectocide levels at the site of parasite location remain efficacious.

ML compounds have a broad spectrum of activity against adult and larval forms of many gastrointestinal nematodes in cattle. However, this pattern of efficacy varies among different nematode parasites and *Cooperia* spp. seem to be the dose-limiting nematode parasite for these compounds. While the reason for this differential susceptibility among different nematodes remains unknown, the evaluation of ML concentrations in different sections of the gastrointestinal tract provided some useful information. High MLs concentrations were detected in both abomasal and small intestinal mucosal tissues up to 48–58 days postsubcutaneous treatment to cattle, which accounted for the large AUC values observed in both target sites. These concentration profiles obtained in the mucosal tissue were greater than those obtained in abomasal and intestinal fluids. Only low MLs concentrations (below 1 ng/ml) were found in abomasal fluid following its subcutaneous administration to cattle, which is in agreement with the data previously reported for ivermectin (Bogan and McKellar, 1988), where the drug was not detected in abomasal fluid, even when administered at 10 times the therapeutic dose in sheep. The greater MLs concentrations observed in the small intestinal fluid collected distal of the bile duct, compared with the abomasal fluid, may be associated to the biliary excretion of the drug. The higher lipid composition of the gastrointestinal mucosa compared to the fluids, considered to be a more polar medium, may account for the greater moxidectin availability found in the mucosal tissues. The high availability and extended residence of MLs in the abomasal and intestinal mucosal tissues agree with the reported persistence of the anthelmintic effects of the drug against different nematodes.

After oral administration of MLs to sheep, high drug concentrations were measured at the GI tissues of parasite location (abomasum and small intestine) during 3–4 days posttreatment. These concentration profiles were significantly higher than those measured in the bloodstream. The highest drug levels were measured at the abomasal and intestinal contents. These high concentrations in the GI contents are significantly higher than those obtained when the MLs were administered by the subcutaneous route to cattle (Lifschitz et al., 1999a, 2000). The drug concentrations measured in the abomasal content for the MLs reflects the amount of drug accumulated within the adult *H. contortus*. After the administration of MLs by an enteral route (oral, intraruminal), the access of drug to *H. contortus* was substantially greater than that obtained following subcutaneous administration. This finding may confirm that an advantageous transcuticular pattern of drug uptake may occur for nematodes located at the GI tract, when lipophilic compounds formulated as a drug solution are orally administered, achieving large availability of soluble drug at the GI lumen (Lloberas et al., 2012, 2013). The comparison of ivermectin and moxidectin concentration profiles achieved in plasma, GI target tissues, and *H. contortus* collected from infected animals (oral treatments) is shown in Figure 41.7.

Although differences in feeding mechanisms and location in the digestive tract among different nematodes should be considered to understand the overall action of the ML endectocides, the persistence of the broad-spectrum anthelmintic activity of the drug against adult and immature gastrointestinal parasites is facilitated by its pattern of distribution and prolonged residence in the gastrointestinal tract. The described pharmacokinetic differences between moxidectin and ivermectin combined with some subtle mechanistic pharmacodynamic differences (see Section Mechanism of Action: Ecto–Endoparasiticadal Activity), are likely to be responsible for the observed differences in potency, spectrum, and expression of resistance seen between both molecules belonging to different ML families. The overall comparison of the pharmacological properties of moxidectin and ivermectin is shown in Figure 41.8.

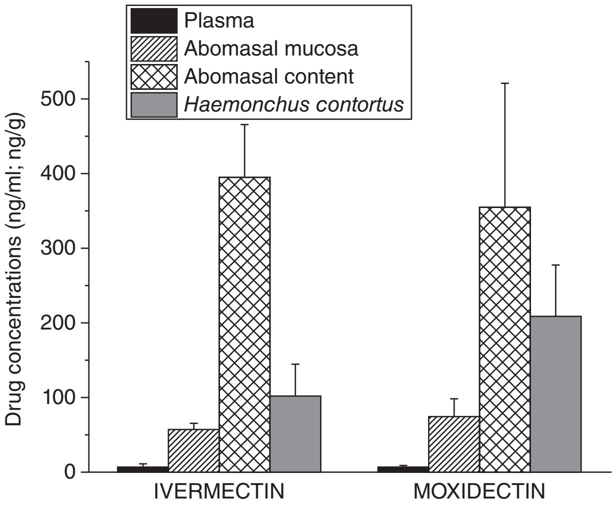


Figure 41.7 Ivermectin and moxidectin concentrations in plasma, gastrointestinal target tissues, and *Haemonchus contortus* measured at 1 day after their intraruminal administration (0.2 mg/kg) to infected sheep. Source: Data from Lifschitz et al., 2013.

ML endectocides are highly effective in eliminating mites and suckling lice species after subcutaneous and topical administration. The pattern of ivermectin and doramectin disposition in skin tissue showed that high concentrations of both molecules (>27 ng/g) are attained during the first 8 days posttreatment (Lifschitz et al., 2000). The sustained presence of high concentrations of ivermectin and doramectin in skin were reflected in prolonged mean residence times (6.8 and 9.3 days, respectively), which may also account for the efficacy of these drugs against single-host ticks. The duration of effective concentrations of endectocide compounds in plasma may be important in the treatment of tick infestations, since this parasite can accumulate lethal drug concentrations through its feeding activity over a period of several days. Drug uptake and efficacy against arthropod ectoparasites can be markedly influenced by parasite feeding habits. Thus, the lower efficacy of the ML against biting lice can be attributed to the lower exposure of this ectoparasite to body fluids containing drug. Moxidectin concentrations greater than 9 ng/g were detected during the first 8 days posttreatment in the skin of treated cattle (Lifschitz et al., 1999a), with a peak concentration of 84.2 ng/g achieved at day 1 postadministration. Drug concentrations in skin declined gradually with time posttreatment, as shown for other tissues, being detectable

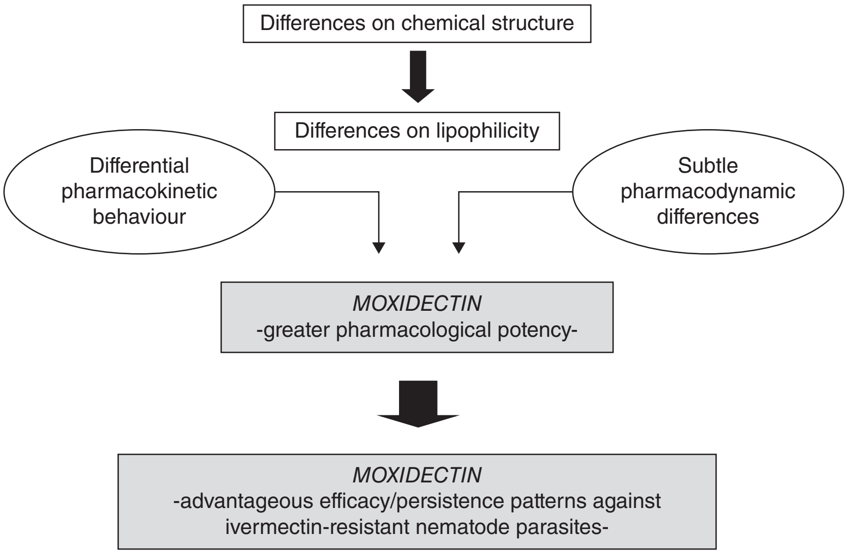

Differences on chemical structure
Differences on lipophilicity
Differential pharmacokinetic behaviour
Subtle pharmacodynamic differences
MOXIDECTIN
-greater pharmacological potency-
MOXIDECTIN
-advantageous efficacy/persistence patterns against ivermectin-resistant nematode parasites-

Figure 41.8 Comparative pharmacological properties for moxidectin and ivermectin. See the text for further explanation.

up to 58 days (>0.2 ng/g). Interestingly, the ML concentration profiles in the skin (dermis and epidermis) were greater than those found in the hypodermal tissue. Blood vessels in the skin are limited to the dermis, which receives the largest vascular supply. The dermis participates in the exchange of compounds between blood and tissues and as a fat reservoir (Monteiro-Riviere, 1991). The arthropod ectoparasites are exposed to systemic agents during feeding; the nature of their food source, and frequency and duration of feeding can have a marked influence in drug uptake and efficacy (Jackson, 1989).

Therapeutic Uses: Animal Species-Specific Considerations

The ML compounds have exceptional potency and broad spectrum of activity against nematodes and arthropods of different animal species. Their lack of efficacy against trematodes and cestodes may be related to the deficiency of ML-specific binding sites in these parasites (Shoop et al., 1995b). As opposed to benzimidazole anthelmintics, the MLs do not possess ovicidal activity. Compounds from both endectocide families share a similar broad spectrum against endo- and ectoparasites; however, some differences in activity against specific parasites may be observed among different ML molecules. A 0.2 mg/kg dose rate is required for avermectin and milbemycin compounds to eliminate their dose-limiting nematode species such as *Cooperia* spp. and *Nematodirus* spp., and to achieve the full broad-spectrum antiparasitic claim in ruminants (Shoop et al., 1995a). Extensive and detailed information on the spectrum of ecto–endoparasitic activity and on the preventive and therapeutic uses of the ML compounds in different animal species has been thoroughly reviewed elsewhere (Armour et al., 1980; Campbell, 1989; McKellar and Benchaoui, 1996; Vercruysse and Rew, 2002). Readers are also referred to the eighth edition of this text (Reinemeyer and Courtney, 2001) for additional specific information regarding the MLs spectrum of antiparasitic activity. However, some overall concepts on the recommended use of the ML in different animal species are summarized below.

Ruminants

Macrocyclic lactones compounds are available to be administered to ruminants by subcutaneous (cattle, sheep), oral (sheep and goats) (0.2 mg/kg), and topical (cattle) (0.5 mg/kg) formulations. ML are highly effective against immature and adult stages of different gastrointestinal nematode species from ruminants such as *Ostertagia* spp. (including inhibited EL_4 in cattle), *Haemonchus* spp., *Cooperia* spp., *Nematodirus* spp., *Trichostrongylus* spp., *Strongyloides* spp., *Bunostomum* spp., *Trichuris* spp., *Oesophagostomum* spp., and *Chabertia ovina*. MLs also possess high efficacy against lung worms (*Dictyocaulus* spp.) in sheep and cattle. The MLs are active against the most of the nematode parasites affecting goats. However, the low systemic availability of some ML compounds obtained in goats (Scott et al., 1990; Alvinerie et al., 1999b) has restricted their use to extralabel prescriptions given at higher dose rates (0.3–0.4 mg/kg) for both oral and parenteral preparations.

Arthropod parasites controlled by MLs include mites (*Sarcoptes bovis, Psoroptes ovis*), oestrid larvae (*Hypoderma bovis, H. lineatum, Oestrus ovis*), and sucking lice (Linognathus vituli, L. pedalis, and *Hematopinus eurysternus*). Ivermectin, abamectin, and doramectin are highly effective against *Dermatobia hominis* infestations. While ivermectin and abamectin aid in the control of the screw worm *Cochliomya hominivorax*, doramectin has demonstrated a distinctive 100% protection for 21 days postinfection in calves (Moya-Borja et al., 1997). The specific distribution of the MLs in the different skin layers and their prolonged residence agree with its pattern of ectoparasiticide activity. The lower efficacy of the ML subcutaneously administered against biting (*Damalinia spp.*) compared to sucking lice, may be associated with the superficial feeding of these parasites, particularly on sloughed epidermal cells, which may contain low drug concentrations. The pour-on formulations are highly effective to control both sucking and biting lice. MLs are slightly less effective in controlling the sheep ked (*Melophagus ovinus*). MLs are highly efficacious against cattle ticks (*Rhipicephalus microplus*). Ticks are exposed to systemic agents as endectocides during feeding; therefore the frequency and duration of feeding may have a marked influence in drug uptake and efficacy. The ML induces either ticks mortality, lower engorgement, or fewer viable eggs. The injectable ivermectin preparation (1%) shows activity against ticks during 7 days posttreatment. Moxidectin and doramectin provide effective control of ticks for 28 days posttreatment, which is extended up to 75 days in the case of the long-acting 3.15% ivermectin formulations. The ML endectocides contribute to the control of *Haematobia irritans* (horn fly) in cattle. These compounds kill the adult flies and inhibit larval development in cattle dung. Development of the *H. irritans* fly may be reduced over 4 weeks after treatment with some ML compounds in cattle.

The concept of antiparasitic persistence acquired great clinical significance after the discovery and commercial development of the ML endectocide compounds. The intrinsic properties of the ML and/or their pharmacotechnical preparations allow a sustainable persistence of the active drug in the animal body and therefore prevent the establishment of new nematode burdens for several days after treatment. Large variations in the duration of the ML persistence of antiparasitic activity have been reported, particularly in cattle. The high variability is based on the parasites genera involved, the type of formulation used, different host-related factors, and the design of the experimental trials. For instance, the classic 1% ivermectin and abamectin formulations administered subcutaneously to cattle showed a persistent activity against *Cooperia* spp., *Ostertagia* spp., and *Dictyocaulus* spp. during 7, 14, and 28 days posttreatment. Longer protection periods against the same nematode parasites have been reported for moxidectin and doramectin. In fact, doramectin showed a protection period between 21 and 35 days against *O. ostertagi* and 14–28 days for *Cooperia* spp. (Vercruysse et al., 2000). Moxidectin provides a protection against *Ostertagia* spp. and *Dictyocaulus* spp. during 35 and 42 days, respectively (Vercruysse et al., 1997). The ML pour-on formulations protect against nematode reinfection during the same period as that obtained after the subcutaneous treatments in cattle. The available ivermectin sustained-released bolus and the more recently introduced highly concentrated long-acting formulations (ivermectin and moxidectin) recommended for use at higher dose rates (between 0.63 and 1 mg/kg, respectively) dramatically increase the protection periods (between 60 and up to 150 days) against cattle endoparasites.

Swine

The MLs are licensed to be used in pigs by subcutaneous (ivermectin, moxidectin), intramuscular (doramectin) administration at 0.3 mg/kg, and oral feed formulation (ivermectin) at 0.1 mg/kg over 7 days to control endo- and ectoparasites. These compounds are active against immature and adult stages of *Ascaris suum*, *Hyostrongylus rubidus*, *Strongyloides ransomi*, *Oesophagostomum* spp., *Metastrongylus* spp., *Stephanurus dentatus*, and the intestinal (but not muscular) stages of *Trichinella spiralis*. Ivermectin efficacy against *Trichuris suis* is highly variable (between 60 and 95%). The MLs present high activity against the most common ectoparasites affecting swine herds: sucking lice (*H. suis*) and mange mites (*S. scabiei var. suis).*

Horses

Ivermectin (0.2 mg/kg) and moxidectin (0.4 mg/kg) are the only MLs available to be used in horses by either oral paste or gel formulations. Both compounds are highly effective against *Parascaris equorum, Oxyuris equi,* stomach worms *(Draschia megastoma, Habronema* spp.*, Trichostrongylus axei), Strongyloides westeri,* and lungworms *(D. arnfieldi).* Both compounds are effective to control adult and larval stages of large strongyles *(Strongylus vulgaris, S. equinus, S. edentatus),* adult small stronglyles *(Cyathostomes), Gasterophilus nasalis,* and *Onchocerca* spp. *microfilariae.* Consistent with the observed longer residence time in the bloodstream and tissues (Perez et al., 1999), moxidectin shows an extended period of protection against most of the target parasites in horses compared to ivermectin (Demeulenaere et al., 1997). The most important differences in the comparative efficacy of ivermectin and moxidectin in horses have been observed against mucosal larvae of C*yathostomes* and G*asterophilus* larvae. Ivermectin has lower efficacy than moxidectin against encysted *Cyathostomes* (Xiao et al., 1994; Monahan et al., 1996). However, while ivermectin is highly active (>99%) against all instars of *Gasterophilus intestinalis*, moxidectin shows a variable activity that ranges between 20 and 99% of efficacy when administered at recommended doses.

Dogs and Cats

The overall efficacy and safety of the ML designed for other species should not be assumed for dogs. It is now well known that Collie dogs and other dog breeds are particularly sensitivity to ivermectin (see Section Safety and Toxicity). Thus, ivermectin is licensed for specific therapeutic uses in dogs and cats. The available preparations are chewable formulations containing ivermectin to be administered at low doses 0.006 mg/kg (dogs) or 0.024 mg/kg (cats) as preventive against dirofilariasis. Ivermectin is effective against third and fourth stages of *Dirofilaria immitis* (Campbell, 1989). Ivermectin and moxidectin approved formulations have limited-spectrum activity against gastrointestinal nematodes in dogs and cats. However, ivermectin administered orally at higher doses (between 0.05 mg/kg and 0.2 mg/kg) may reach good efficacy against various nematode parasites (*Ancylostoma caninum*, *Trichuris vulpis, Toxocara canis,* and *Capillaria aerophila*). Different studies have shown that two ivermectin subcutaneous doses (0.2 mg/kg) are enough to clear otodectic, sarcoptic, and notoedric mange infestations (Campbell, 1989). Ivermectin is also used in the control of demodectic mange infestations in dogs at higher dosages and prolonged regimens. However, the therapeutic uses of ivermectin in dogs and cats, apart from the prevention of *D. immitis*, should be considered as an extralabel use.

Milbemycin oxime administered at 0.5 mg/kg is effective against developmental stages of *D. immitis, T. canis, T. leonina, A. caninum,* and *T. vulpis*. Moxidectin has been introduced as a tablet (0.003 mg/kg) and long-acting sustained release injectable formulation (0.17 mg/kg) to prevent *D. immitis* infection. This injectable formulation is also useful to control *A. caninum*. Selamectin, a new avermectin compound (see Figure 41.1) introduced as a topical formulation for dogs and cats, is active against different endo- and ectoparasites at 6 mg/kg. A greater selamectin systemic availability was observed in cats compared to that obtained in dogs (Sarasola et al., 2002). It is highly efficacious against infective stages of heartworm (*D. immitis*), *T. canis*, *T. leonina*, *A. tubaeforme,* and *T. cati.* Selamectin is also effective against ectoparasites such as fleas (*Ctenocephalides cati)*, mites (*Otodectes cynotis, S. scabiei*), and ticks (*R. sanguineous* and *Dermacentor variabilis*).

Resistance

The antiparasitic strategies based almost exclusively on chemical control have been threatened by the emergence of nematode parasites resistant to the various anthelmintic chemical classes, including now the ML compounds. The increasing selection pressure on the gastrointestinal nematodes due to the high frequency of ML treatments was relevant for the development of resistance to these compounds. Changes in the glutamate-gated chloride channels structure and an increased expression of different proteins involved in drug efflux (P-GP) have been postulated as the main mechanism of resistance to MLs in nematodes (see Section Mechanism of Action: Ecto–Endoparasiticadal Activity). It has been shown that *H. contortus* resistant to ivermectin has an increased level of P-GP expression and that the coapplication of verapamil (a multidrug resistance (MDR)-reversing agent) increased the efficacy of ivermectin and moxidectin against resistant strains of *H. contortus* (reviewed by Prichard, 2002). The up-regulation of P-GP in *H. contortus* recovered at 1 day postadministration of ivermectin was reported earlier; this also occurs to a lesser degree after the moxidectin administration (Prichard and Roulet, 2007). It has also shown that the ivermectin treatment significantly increased the P-GP2 expression in resistant *H. contortus* recovered from treated lambs 0.5 and 1 days posttreatment compared to worms recovered from untreated animals. The up-regulation of P-GP is in agreement with the highest ivermectin concentrations measured in *H. contortus* (Lloberas et al., 2013). Similar results were obtained in cattle parasites such us *C. oncophora* where the P-GP 11 was up-regulated upon MLs exposure (De Graef et al., 2013).

Initially, resistance to the ML (largely to ivermectin) was observed in sheep and goats nematodes, particularly in *H. contortus*. However, the phenomenon has been widespread and other nematode species from small ruminants have developed resistance to the ML compounds. Field strains of *Teladorsagia circumcincta* and *T. colubriformis* resistant to ivermectin has been identified around the world. In addition, the intensive global use of the different ML compounds over the last 25 years (since the introduction of ivermectin in the early 1980s), has resulted in the emergence of ML resistance in cattle nematodes. Different field strains of *Cooperia* spp., the dose-limiting parasite for MLs, *Trichostrongylus* spp., and Ostertagia spp. have been identified as resistant to the ML compounds (Vermunt et al., 1995; Coles et al., 1998; Fiel et al., 2001; Demeler et al., 2009; Gasbarre et al., 2009; Bartley et al., 2012a). It should be noted that moxidectin conserves activity against some ivermectin-resistant isolates, which is probably due to its differential kinetic and dynamic behavior compared to ivermectin (see Figure 41.8).

A decrease in the selection pressure, reducing the number of treatments per year, together with an integrated antiparasitic control strategy, is urgently required to prolong the usefulness of the broad-spectrum parasite control of MLs in food-animal production. Detailed information on the parasite resistance against ML compounds in ruminant species has been reviewed by Prichard (2002), Wolstenholme et al. (2004), and Wolstenholme and Kaplan (2012).

Despite the widespread resistance of different equine parasites to different anthelmintics, and taking into account that ivermectin has been used in horses for 20 years, there are still no reports of *Cyathostomes* resistance (Kaplan, 2002). ML resistance has been described in *Oxiuris equi* (Wolf et al., 2014) and *Parascaris equi* (Bishop et al., 2014). Considering that ivermectin is the single most commonly used anthelmintic in horses, it seems that emergence of resistance would be inevitable. There are no reports of resistant heartworms, in spite of the fact that MLs have been extensively used to prevent infections in dogs on a monthly basis.

Several reports on ectoparasites resistance to ivermectin have been published. Resistant populations of the cattle tick *Rhipicephalus* (*Boophilus*) *microplus* were identified in Brazil and Mexico (Martins and Furlong, 2001; Klafke et al., 2006; Perez-Cogollo et al., 2010). It seems that some ABC transporters, such as P-GP, may participate as a resistance mechanism to ivermectin in ticks (Pohl et al., 2011).

Pharmacological Interaction with ABC Transporters: Practical Implications

Different in vitro and ex vivo methods have been reported to characterize the interactions between MLs and ABC transporters. It is interesting to note that moxidectin appears to have different P-GP efflux potential, with a half-maximal inhibitory effect (IC_{50}) approximately 10 times higher than that reported for ivermectin (Griffin et al., 2005; Lespine et al., 2007). P-GP may be involved in the ivermectin efflux at low drug concentration, whereas at the higher micromolar concentration range (which may saturate P-GP function), MRP1, and to a lesser extent the MRP2 and MRP3, efflux proteins could participate in ivermectin transport across physiological barriers (Lespine et al., 2006b). The interaction of MLs with BCRP was described (Muenster et al., 2008). It seems that ivermectin is a relatively potent P-GP inhibitor and rather weak BCRP inhibitor. Moxidectin was identified as a BCRP substrate, and the BCRP-mediated secretion of moxidectin in milk was demonstrated using cellular transport assays and pharmacokinetic studies in *BCRP1* (−/−) and wild type mice (Pérez et al., 2009). The involvement of ABC transporters in the milk secretion of MLs may play a relevant role in the clinical use of these compounds in lactating animals due to the presence of residues in milk and derived products.

Important changes to the plasma disposition of the MLs have been observed when these compounds are coadministered with P-GP modulators. The oral coadministration of verapamil with ivermectin induced a significant increase in the ivermectin absorption in sheep (Molento et al., 2004). In lambs the coadministration of quercetin, a derived flavonoid present in vegetables, with moxidectin produced a significant increase on moxidectin plasma concentrations (Dupuy et al., 2003). A significant increment in the systemic availability of ivermectin was obtained after its administration together with the antifungal drugs itraconazole (Ballent et al., 2007) and ketoconazole in sheep (Alvinerie et al., 2008) and dogs (Hugnet et al., 2007). The influence of loperamide (LPM) on the plasma disposition and fecal excretion of moxidectin after intravenous and subcutaneous administration was studied in cattle (Lifschitz et al., 2002b). The modulation of the activity of P-GP has been assayed as a pharmacology-based strategy not only to increase the systemic availability of the MLs in the host animal but also to improve their clinical efficacy. The modulation of P-GP with verapamil, ketoconazole, and pluronic 85 increased the in vitro activity of ivermectin against ivermectin-sensitive and resistant larvae of *T. circumcincta* and *H. contortus* (Bartley et al., 2009). Further scientific support was obtained by demonstrating that verapamil increased the in vitro ivermectin activity against susceptible and resistant isolates of *Cooperia spp.* (Demeler et al., 2013b). Although a modification in MLs activity after P-GP modulation was confirmed in vitro, in vivo trials performed under field conditions are necessary to evaluate the clinical impact of the P-GP inhibition. The enhanced sensitivity of resistant larvae to ivermectin obtained after its coincubation with pluronic 85 did not correlate with their in vivo coadministration to sheep. In the in vivo trial, the presence of pluronic 85 did not improve the efficacy against resistant *H. contortus* (Bartley et al., 2012b). The efficacy of both ivermectin and moxidectin against resistant *Cooperia spp.* in a cattle field trial tended to increase after their coadministration with loperamide, a P-GP modulator (Lifschitz et al., 2010a). This was further corroborated as a significant increase in IVM efficacy against resistant nematodes in sheep together with an enhancement of the systemic availability of the antiparasitic compound in the presence of the P-GP inhibitor (Lifschitz et al., 2010b). Thus, there is evidence that the P-GP-mediated drug–drug interaction increases the ivermectin systemic exposure in the host and it may also decrease the P-GP-mediated efflux transport over-expressed in target resistant nematodes. Table 41.1 summarizes the different in vitro/in vivo assays performed to study the modulation of MLs antiparasitic activity by inhibitors of P-GP. The practical implications of this drug interaction should be carefully investigated regarding the potential side effects induced by the P-GP modulating agents (see Chapter 50 for further discussion). However, the modulation of either P-GP or other proteins involved in

Table 41.1 Influence of P-glycoprotein modulation on the activity/efficacy of different macrocyclic lactones (MLs) against ivermectin susceptible and resistant nematode strains. Source: Adapted from Lifschitz et al., 2012.

ML compound/ P-GP modulator	Parasite/animal species	Modification of activity	Reference
IVM + verapamil MXD + verapamil	*H. contortus* in jirds	Enhanced efficacy (from 1.71 to 2.69-fold)	Molento and Prichard, 1999
IVM + ketoconazole IVM + PSC833 IVM + verapamil IVM + pluronic85	IVM-sensitive and resistant larvae of *T. circumcincta* and *H. contortus*	Increment on IVM in vitro activity (larval feeding inhibition test) from 10 up to 77-fold	Bartley et al., 2009
IVM + verapamil	IVM-sensitive and resistant *Cooperia spp.* larvae	Enhancement on IVM in vitro activity (larval development and migration tests) between 10 and 100-fold	Demeler et al., 2009
IVM + pluronic 85	Resistant *H. contortus* in sheep	No changes on efficacy compared to the IVM alone treatment	Bartley et al., 2012b
IVM + loperamide MXD + loperamide	Resistant *Cooperia spp.* in cattle	Increment on efficacy (FECRT) from 23 to 50% (IVM) and from 69 to 87% (MXD)	Lifschitz et al., 2010a
IVM + loperamide	Resistant *H. contortus* and *T. colubriformis* in sheep	Enhanced IVM efficacy (adult nematode counts) from 0 to 72.5% (*H. contortus*) and from 77.9 to 96.3% (*T. colubriformis*)	Lifschitz et al., 2010b
IVM + verapamil IVM + Vincristine IVM + Vinblastine	IVM sensitive wild-type and resistant strains of *Caenorhabditis elegans*	Significant changes on motility and pharyngeal pumping in the IVM-resistant strain	Ardelli and Prichard, 2013

IVM, ivermectin; MXD, moxidectin; FECRT, fecal egg counts reduction test.

drug transport may be a valid pharmacological approach to improve the activity and extend the lifespan of the MLs in veterinary medicine.

Available Pharmaceutical Preparations

Different delivery systems have been developed to administer ML compounds to different animal species. The available preparations include injectable solutions, oral drenches, tablets, pastes, in feed premix, pour-on formulations, and a sustained released bolus (ivermectin). An oral paste containing 1.87% of ivermectin is available for horses. In-feed formulation of ivermectin for pigs is recommended over 7 consecutive days at 0.1 mg/kg per day. Ivermectin is also formulated in a micellar solution for oral drench use in sheep and goats, and as tablet and chewable preparations for oral administration in dogs and cats. The pioneer and worldwide-distributed ivermectin 1% injectable formulation for subcutaneous administration (0.2 mg/kg)in sheep and cattle (Ivomec®, Merial Ltd) is formulated in propylene glycol (60%) and glycerol formal (40%). A topical ivermectin formulation is also commercialized to use against the whole range of ecto- and endoparasites of cattle at 0.5 mg/kg.

Pharmaceutical technology has been applied to develop different drug formulations and delivery systems to optimize the pharmacological potency of ivermectin and other ML endectocides currently available. It has been confirmed that extended residence times of ivermectin in plasma and target tissues and the prolonged persistence of its anthelmintic activity are obtained following the administration of an alternative oil-based formulation to cattle, compared to the standard preparation (Lifschitz et al., 1999b). Recently developed injectable long-acting formulations for cattle are essentially oil based or thixotropic (3.15%) preparations that account for a slow absorption process from the subcutaneous space and a long persistence of active ivermectin concentrations in the bloodstream and target tissues (Lifschitz et al., 1999b, 2007). This 3.15% ivermectin formulation (Ivomec Gold®, Merial Ltd) given at higher dose rates (0.63 mg/kg) has persistent efficacy against cattle gastrointestinal and lung nematodes for up to 77 days and ticks for up to 75 days. Following the same concept of the extended antiparasitic persistence, other similar (generic) highly concentrated (3.15% and 3.50%) ivermectin preparations have been recently introduced into the market for use in cattle in some countries. Another recently developed technology incorporates eprinomectin in a long-acting preparation based on poly (lactic-coglycolic) acid copolymer, which extend its persistent activity against nematodes for up to 120 days (Marley and Tejwani, 2005). The development of sustained-release bolus technology for the administration of antiparasitic drugs was considered an important advance for parasite control. The ivermectin sustained release device is an osmotic bolus delivering

12.7 mg of ivermectin per day over 135 days postadministration to cattle. The excellent release performance of an ivermectin sustained-release bolus was confirmed measuring the plasma and fecal concentration profiles up to 160 days postadministration in cattle; 90% of the total dose released from the bolus was excreted in feces (Alvinerie et al., 1998b).

Controlled-release capsules providing 1.6 mg of ivermectin per day over 100 days have been commercialized for use in sheep. More recently, novel delivery technology has been applied to develop a sequential-release formulation for use in sheep, which delivers ivermectin and albendazole alternately (Gogolewsky et al., 2005). This ivermectin/albendazole sequential delivery (10 days of ivermectin followed by 20 days of albendazole release, with the same alternating release cycle to cover 100 days posttreatment) has been designed to assure the exposure of incoming parasite larvae to two chemicals with different mode of action, which could delay the development of resistance to each of the anthelmintic agents.

Doramectin is available in an oil-based formulation containing sesame oil/ethyl oleate (90 : 10) to be administered by the subcutaneous and intramuscular routes to cattle. A doramectin pour-on preparation is licensed to be used in cattle at 0.5 mg/kg. Recently, a concentrated long-acting formulation of doramectin (3.5%) was launched to be used in cattle at 700 μg/kg. This formulation supplies an extended persistence of antiparasitic action against nematodes and the main ectoparasites affecting cattle.

Moxidectin is available to be administered as injectable, pour-on (cattle) and oral (sheep and horses) formulations. A 1% nonaqueous moxidectin formulation is currently available for subcutaneous injection in cattle, sheep (0.2 mg/kg), and pigs (0.3 mg/kg). Novel moxidectin long-acting preparations for use in sheep (2%) and cattle (10%) are now available for use at higher dose rates (1 mg/kg) to cover extended antiparasitic protection periods (between 60 and 150 days). A long-acting sustained-release injectable formulation for dogs (0.17 mg/kg) designed to prevent *D. immitis* infection is currently available. A moxidectin gel preparation to be administered orally at 0.4 mg/kg to horses is also available in the veterinary pharmaceutical market.

Eprinomectin is approved as a topical formulation for use in beef and dairy cattle. Both the eprinomectin and moxidectin pour-on formulations are now indicated in lactating dairy cows with zero milk withdrawal time in some countries. Selamectin is formulated as a solution (6 and 12%) in an isopropyl alcohol/dipropylene glycol methyl-ether vehicle to be used in dogs and cats at a dose range between 6 and 12 mg/kg (Bishop et al., 2000).

Drug and Host-Related Factors Affecting Pharmacokinetics and Efficacy in Ruminants

As described in this chapter, the MLs are highly lipophilic substances that dissolve in most organic solvents. They are large molecules and despite possessing two sugar rings and two hydroxyl groups (avermectins) are relatively insoluble in water. Moxidectin solubility in water is greater than that of ivermectin and doramectin. These and other physicochemical differences between the ML molecules may account for differences in formulation flexibility and in their resultant kinetic behavior. The vehicle in which the endectocide compounds are formulated may play a rate-limiting role in their absorption kinetics and resultant plasma availability. The plasma profiles of ivermectin (Lo et al., 1985) and doramectin (Wicks et al., 1993) in cattle have been shown to be substantially affected by the composition of the administered formulation. Following parenteral administration, the low solubility of ivermectin and doramectin in water, and its deposition in subcutaneous tissue, favor a slow absorption from the injection site and provide prolonged duration in the bloodstream. Lanusse and coworkers (1997) characterized the comparative plasma disposition kinetics of ivermectin, moxidectin, and doramectin after their subcutaneous administration of the formulations commercially available for administration to cattle. While ivermectin was administered as the nonaqueous (60% propylene glycol/40% glycerol formal) preparation, an oil-based formulation of doramectin containing sesame oil/ethyl oleate (90 : 10) was administered to the experimental animals. The absorption of moxidectin, administered as an aqueous-based solution, from the site of subcutaneous injection was significantly faster than those of ivermectin and doramectin. The low solubility and the deposition of the ivermectin and doramectin nonaqueous preparations in the subcutaneous tissues favor a slow absorption from the site of injection, which may account for their prolonged residence in the bloodstream in cattle.

Several generic formulations of ivermectin have been introduced into the pharmaceutical market in different regions of the world after the expiration of the original patent of the first approved (innovator) ivermectin formulation (Ivomec®, Merial). Most of the available ivermectin generic preparations (some of them are now very well established in the pharmaceutical market) contain basically the same vehicle composition used in the innovator ivermectin formulation, but information on the comparative kinetic behavior of generic preparations in standardized pharmacokinetic trials is scarce. Lifschitz et al. (2004) found marked differences on the absorption kinetics of ivermectin given by subcutaneous injection

Table 41.2 Drug and host-related factors affecting the kinetics and efficacy of the macrocyclic lactones in ruminants

Pharmacokinetic modifications ↓ Changes in drug concentrations in target tissues ↓ Modified efficacy and/or antiparasitic persistence	Type of drug formulation Route of administration Licking behavior (topical treatment) Animal species Animal breed Nutritional condition and/or body composition Type of diet and/or level of feed intake (oral treatment) Parasitism and/or modified gastrointestinal transit time P-GP-mediated changes on drug absorption and/or excretion

The drug and host-related factors listed on the right may induce pharmacokinetic modifications resulting in modified drug efficacy/persistence. See the text for further explanation.

to cattle as four different generic (1%) formulations. The composition and quality of the vehicles and/or excipients used in the pharmacotechnical elaboration of ML formulations may be relevant to its pharmacokinetic behavior. The differences observed on the systemic availability and drug disposition kinetics among generic formulations may be reflected in the efficacy and persistence of their antiparasitic activity. Since the direct relationship between time of persistence of drug concentrations and extended efficacy against endo- and ectoparasites has been demonstrated, slight differences in formulation account for changes to the plasma kinetics and exposure of target parasites to active drug concentrations. This study was useful to build up a "conscience" on the relevance that the switchability among generic ML formulations might have on parasite control. Considering the necessary precautions to be adopted to avoid/delay the development of ML resistance in cattle nematode parasites, standardized quality control of generic formulations may greatly contribute to optimized drug use.

A number of factors affecting drug kinetics and the persistence of the antiparasitic efficacy of the ML have been identified. Among them are the animal species/breed, level of feed intake, nutritional status/body composition, influence of parasitism, formulation of dosage form, route of administration, etc. (reviewed by Lanusse and Prichard, 1993; McKellar and Benchaoui, 1996; Hennessy and Alvinerie, 2002) (Table 41.2). A series of relevant studies to compare the plasma profiles of ML in different species have been reported (Alvinerie and Galtier, 1997). From those comparisons, the consistently lower systemic availability of moxidectin (oral treatment) and ivermectin (subcutaneous treatment) observed in goats compared to sheep, emerges as a relevant finding. The influence of the route of administration on the plasma availability of the MLs has been extensively studied in ruminant species. For instance, both ivermectin and moxidectin are markedly less absorbed following oral administration to sheep compared to their subcutaneous injection. The reduced systemic availability observed following oral treatment accounted for lower milk residues excretion in orally treated dairy sheep compared to those observed after the injectable treatment (Imperiale et al., 2004b), which may be considered advantageous in required withdrawal times extension.

In the past, shortly after introduction of ivermectin into the market, nematode susceptibility to ivermectin was high and equivalent efficacy patterns were observed against abomasal parasites following its parenteral and oral administrations. However, after the oral administration of MLs to lambs infected with resistant parasites, the efficacy was higher compared to that obtained after subcutaneous treatment (Gopal et al., 2001; Alka et al., 2004; Lloberas et al., 2012). Similar results were obtained in cattle, where the oral administration of moxidectin achieved a higher efficacy against resistant nematodes compared to the subcutaneous treatment (Leathwick and Miller, 2013). The enhanced drug concentrations measured at the abomasal content after the oral administration of MLs, accounted for the greater amount of drug recovered from nematodes such as *H. contortus* (Figure 41.9). These high drug concentrations recovered in the gastrointestinal tract during the first 2–3 days after oral treatment may have a relevant effect against resistant worms, as it is of great importance to induce the pharmacological action at the target site. The increment on drug exposure obtained after the oral treatment (compared to the parenteral administration) may be useful to kill heterozygous resistant parasites present in the earliest phases of the development of resistance.

The ML has been traditionally administered by subcutaneous injection to cattle. However, formulations for their topical administration (pour-on) are currently marketed worldwide. Some practical advantages of topical compared to other routes of administration have accounted for its great acceptance for parasite control in cattle. Some information is available on the plasma kinetic behavior of avermectins given topically to cattle (Gayrard et al., 1999). The plasma and tissue disposition kinetics of moxidectin and doramectin (Sallovitz et al., 2003, 2005) administered as a pour-on to cattle has been characterized. Lower moxidectin and doramectin plasma concentrations were measured following topical

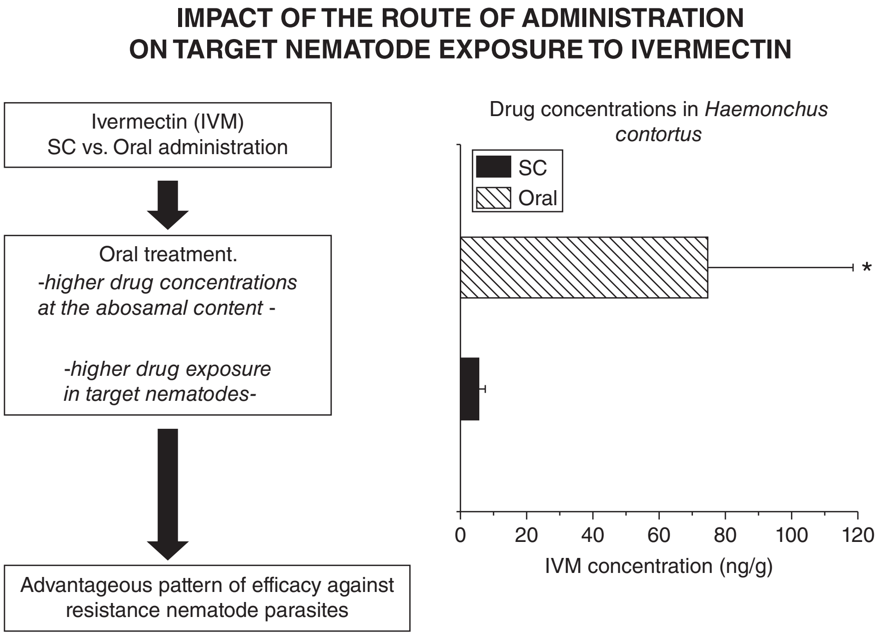
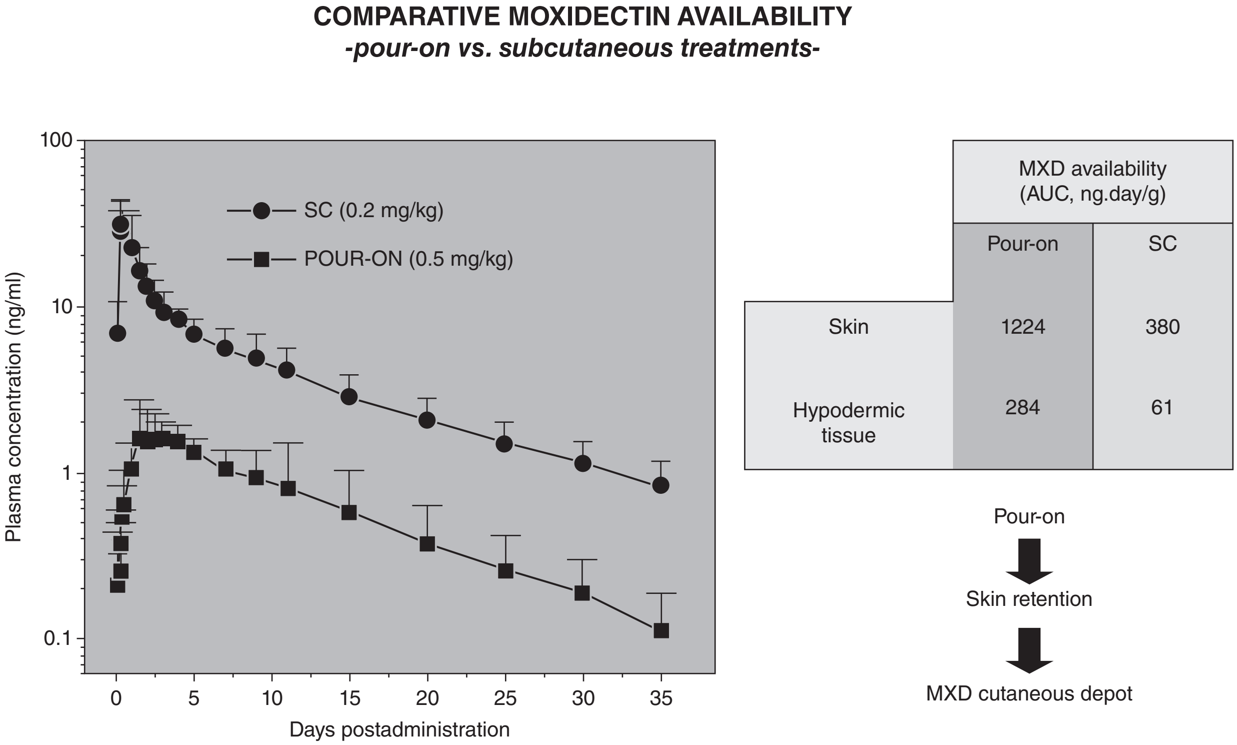


Figure 41.9 Influence of the route of administration on ivermectin exposure to target nematode parasites. Source: Data from Lloberas et al., 2012. The plot on the right side shows the ivermectin concentrations at day 3 posttreatment.

treatment compared to the subcutaneous injection of both compounds in cattle in spite of the use of a 2.5-fold higher dose rate for the pour-on administration (Figure 41.10). The absorption rate of topically administered moxidectin and its plasma availability were significantly higher in Holstein compared to Aberdeen Angus calves (Sallovitz et al., 2002), which may have considerable practical implications in terms of drug activity/persistence. Topically administered moxidectin is extensively distributed to different target tissues, including gastrointestinal mucosas, lungs, and different dermal layers. A cutaneous depot of the topically administered drug accounts for slow and sustained absorption, and agrees with the high moxidectin and doramectin



	MXD availability (AUC, ng.day/g)	
	Pour-on	SC
Skin	1224	380
Hypodermic tissue	284	61

Figure 41.10 Comparative plasma concentration profiles and total drug (expressed as AUC values) recovered in skin and hypodermic tissue after the subcutaneous (SC) and pour-on administrations of moxidectin (MXD) at recommended doses to cattle. The data illustrate the cutaneous depot of MXD after topical treatment. Source: Data from Lifschitz et al., 1999a; Sallovitz et al., 2003.

concentrations measured in the epidermis and dermis of the skin from different anatomical regions.

It has been shown (Laffont et al., 2001) that the plasma and fecal disposition of ivermectin topically administered to cattle is markedly influenced by the natural licking behavior of treated animals. Higher and more variable systemic availability of ivermectin was observed in licker cattle, compared to animals whose licking behavior was prevented. Sallovitz et al. (2005) showed that a 2-day long licking prevention period after the pour-on treatment was sufficient to significantly reduce the doramectin concentrations measured in plasma during the first 9 days posttreatment. The animals in the free-licking group ingested topically administered doramectin by self and/or allolicking, which accounted for the higher peak concentration observed in plasma as well as for the enhanced drug availability compared to the licking-restricted animals. Laffont et al. (2003) demonstrated that after the topical administration of ivermectin to cattle, drug absorption through the gastrointestinal tract due to licking-induced oral ingestion is more important than the transdermal absorption in determining drug systemic concentrations. Thus, a more complete absorption of the MLs in the gastrointestinal tract following oral ingestion of topically administered formulation, compared to cutaneous permeation, considered as the only absorption process in nonlicking animals, would explain the enhanced plasma profiles measured in calves allowed to lick freely. Licking prevention during 10 days posttreatment permitted the identification of marked changes on the doramectin concentration profiles both systemically and, much more importantly, in the gastrointestinal tract. Doramectin concentrations in luminal contents from the intestine were markedly higher in free-licking cattle (Figure 41.11). Doramectin concentrations were higher in the fluid contents than in the mucosas in the licking group. Conversely, in licking-restricted calves, doramectin concentrations were higher in the mucosal tissue than in luminal content of the different gastrointestinal sections. These observations in nonlicking calves are in agreement with the data reported by Lifschitz et al. (2000) after the subcutaneous administration of doramectin, where after absorption into the systemic circulation, the drug can reach the gastrointestinal lumen by a distribution exchange between the bloodstream and the digestive compartment, which must occur through the mucosal layer of the intestine.

On the face of the observed variations on antiparasitic response and with the threat of resistance development, the use of pour-on formulations to control endo- and ectoparasites should be carefully considered. As suggested by Bousquet-Mélou et al. (2004), there are many therapeutic, practical, and regulatory issues to be discussed on the basis of the altered pharmacokinetic behavior induced by the natural licking in pour-on treated cattle. When topical ivermectin-treated and untreated heifers were put together after treatment, the licking behavior induced partial efficacy in untreated animals. Therefore the use of pour-on formulation to develop the selective treatment strategy should be carefully evaluated as the untreated animals may be underexposed to the drug transferred from the treated animals (Bousquet-Mélou et al., 2011). It has been suggested that the inter- and intraanimal variability associated with licking behavior should be considered as a biological fact influenced by social, nutritional, physiological, pathological, environmental, and management factors. As a consequence, it is recommended that the pharmacoparasitological evaluation of pour-on products

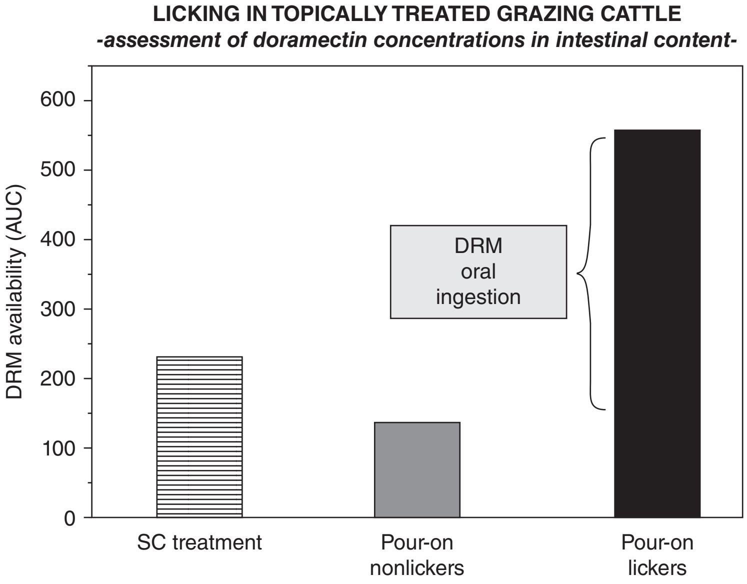


Figure 41.11 Influence of animal's licking behavior on the intestinal availability of topically administered doramectin (DRM) in grazing cattle. The data shows the AUC values obtained for DRM in the duodenal content after its topical administration (0.5 mg/kg) in both nonlickers (licking restricted over 10 days posttreatment) and free-licking calves. The comparison with the values obtained for the subcutaneous treatment (0.2 mg/kg) is included. DRM intestinal availability was significantly higher in licker calves due to licking-induced oral ingestion of the topical formulation. See the text for further explanation. AUC values are expressed in ng.day/ml. Source: Data from Sallovitz et al., 2005.

in cattle be conducted when animals are not prevented from allo- and self-licking (Toutain et al., 2012).

Tissue Residues and Withdrawal Times

Based upon teratogenicity studies in mice and from multigeneration studies in rats, the no-effect level (NOEL) of 0.2 mg/kg/day has been established. Using this NOEL to determine residue tolerances in edible tissues from pharmacokinetic studies (see Chapter 61 for a full discussion of how these values are determined), the current withholding periods for sheep and cattle have been established. However, due to their high potency and elimination through milk, the classic injectable ML formulations are contraindicated for use in animals that produce milk for human consumption. The MLs are highly lipophilic substances extensively distributed to different tissues. The highest tissue residue concentrations in food-producing animals are recovered from the liver and fat. Regulatory agencies (US Food and Drug Administration, European Agency for the Evaluation of Medicinal Products) have established the maximum residue levels (MRL) in target tissues (liver and fat) for some of the ML compounds in sheep and beef cattle. A 100 μg/kg MRL value for ivermectin in liver and fat tissue has been set. The MRL values established for doramectin are 100 μg/kg (fat) and 150 μg/kg (liver). Higher MRL values were proposed for moxidectin in fat (500 μg/kg) and eprinomectin in fat (250 μg/kg) and liver (1500 μg/kg).

Posttreatment withdrawal periods have been established for the commercialized formulations in the different food-animal species. Although the established withdrawal times may vary according to the policies applied by the regulatory agencies in different countries, the classic 1% cattle injectable formulations of abamectin, ivermectin, doramectin, and moxidectin require withdrawal times ranging from 35 up to 50 days. A 120-days period is required to consume tissues from cattle treated with the 3.15% long-acting ivermectin formulation. An 84-days posttreatment period is required to deplete moxidectin below the established tolerance limits for different tissues after its administration as a long-acting preparation (10%) in cattle subcutaneously injected in the base of the ear. Withdrawal times between 10 and 15 days are required to consume meat from sheep treated with the ivermectin oral drench. In the USA, a 45-day (35 days in Europe) withdrawal period is required for beef cattle treated with the available doramectin topical preparation. Required meat withdrawal times for doramectin in pigs parenterally treated are, at present, as follows: 24 days (USA), 28–50 days (Latin America) and 28–77 days (European countries) posttreatment. Detailed information on posttreatment withholding periods for the different ML formulations approved for use in food-producing animals in the USA can be found in the web site for the US Pharmacopeia (www.usp.org). There are no established withdrawal times for goats because MLs are not approved for use in this species. If ivermectin is administered to goats as a single extralabel oral dose of 0.2 to 0.4 mg/kg, evidence has been compiled by the Food Animal Residue Avoidance Databank (FARAD) that suggests a meat withdrawal time of 14 days and a milk withholding time of 9 days, which would be sufficient to avoid potentially harmful residues (Baynes et al., 2000).

Use of all the ML injectable formulations are restricted in lactating dairy animals. However, a pour-on formulation of eprinomectin is approved for topical use in all cattle, including lactating dairy cows, without any milk withdrawal period. A moxidectin pour-on formulation is now available for use in dairy cattle with zero withdrawal time in milk. Additionally, generic ivermectin topical preparations are now approved for use in dairy cattle without milk withdrawal in some specific countries. Milk MRL values have been established for ivermectin (10 μg/kg), eprinomectin (20 μg/kg), and moxidectin (40 μg/kg).

Safety and Toxicity

The ML compounds given at recommended therapeutic doses are highly safe compounds in the majority of the target animal species. These large safety margins are based on the selectivity of their pharmacodynamic action. A GABA-mediated action in the mammalian central nervous system may be responsible for the neurotoxicity observed when large toxic doses of different ML are administered (Lankas and Gordon, 1989). In general, the avermectins have at least a 10-fold safety margin in ruminants, horses, swine, and most dog breeds, except collies and some Australian shepherds. The signs of acute toxicity include depression, ataxia, tremors, salivation, mydriasis, and in severe cases, coma and death. No effect on breeding performance, semen quality, or pregnancy was observed when bulls or cows were treated with ivermectin at 0.4 mg/kg (Campbell and Benz, 1984). Avermectins are safe for use in cows during all stages of pregnancy. A twofold increase in dosage and multiple dosing have not adversely affected spermatogenesis, conception, longevity of gestation, or fetal development. Abamectin is slightly more toxic than ivermectin, with an oral LD_{50} in mice (14–24 mg/kg) lower than that of ivermectin (25–40 mg/kg). Signs of toxicosis were observed in cattle treated subcutaneously with abamectin at levels of 2–8 mg/kg. Abamectin is not recommended for use in calves under 4 months of age (Pulliam and Preston, 1989). No adverse effects were observed in different tolerance studies using up to 25-fold the therapeutic dose of doramectin injectable in cattle. Numerous field trials corroborate that eprinomectin is safe to use in cattle of all breeds and ages. As for the avermectins, milbemycin compounds have a wide therapeutic margin. No adverse effects have been observed in cattle subcutaneously treated with moxidectin up to 10 times the recommended dose (2 mg/kg). Moxidectin is safe in breeding animals. At three times the recommended dose (0.6 mg/kg) no adverse effects on reproductive performance of bulls and pregnant cows were observed. A different degree of neurotoxicosis was observed for moxidectin and ivermectin. Moxidectin has a 2.7-fold lower neurotoxic potential compared to ivermectin (Janko and Geyer, 2013). Moxidectin had a lower brain-to-plasma concentration ratio and entrance into the brain also causes a lower potentiation of GABA action (Ménez et al., 2012). The differences in the accumulation in the brain and in the interaction with GABA receptors may explain the differential neurotoxicity observed for both drugs.

As described in Section Mechanism of Action: Ecto–Endoparasiticadal Activity, P-GP is a transport protein that acts as a multidrug efflux pump reducing intracellular concentration of different drugs, including the MLs. The activity of this transmembrane protein, located in brain capillary endothelial cells, intestinal epithelial cells, biliary canalicular cells, renal proximal tubular epithelial cells, as well as placental and testicular cells, is related to the toxicity of the MLs. Its presence in the blood–brain barrier limits the entry of these antiparasitic molecules into the central nervous system. The accumulation of ivermectin in the brain of genetically engineered P-GP-deficient mice was 87-fold higher compared to normal mice (Schinkel et al., 1994). Certain Collie dogs are more sensitive to high doses of ivermectin than other dogs and develop signs of toxicity with single doses as low as 0.1 mg/kg (Fassler et al., 1991). The higher ivermectin brain concentrations reported in the avermectin-sensitive collies versus nonsensistive dogs may be related to a limited P-GP expression (Mealey et al., 2001; Roulet et al., 2003). A four base-pairs deletion in the *MDR1* (multidrug resistance) gene in sensitive Collie dogs seems to account for altered P-GP synthesis. Dogs homozygous for a mutant allele of *MDR1* have a nonfunctional P-GP (Mealey et al., 2001). A sample population study of 40 Collie dogs in the northwestern United States found 35% to be homozygous for the mutation and 42% to be heterozygous carriers of the mutant allele (Mealey et al., 2002). A gene study of subpopulations has shown that the mutation of the *MDR1* gene can be found in members of breeds other than Collie dogs (Australian Shepherds, English Shepherds, Shetland Sheepdogs, etc.), generally at a much lower frequency (Hadrick et al., 1995). Pharmacogenomics are more fully discussed in Chapter 50.

Animal deficient in P-GP expression in the intestine may also absorb more drug after oral treatment, which may result in higher blood concentrations and enhanced potential for neurotoxicity (Shoop and Soll, 2002). Additionally, limited P-GP expression in the hepatobiliary tract may result in a decrease bile excretion of the ML compounds. Selametin was evaluated in dogs and cats at 10 times the recommended doses and no adverse reactions were observed (Novotny et al., 2000; Krautmann et al., 2000). Selamectin use is also safe in ivermectin-sensitive Collie dogs. No abnormalities were observed in sensitive Collies treated topically with selamectin at 40 mg/kg. Milbemycin oxime is well tolerated in dogs, including Collies. Clinical signs are only seen after the administration of a 10-fold overdose. Overall, as suggested by Shoop and Soll (2002), the wide safety margin is based on the low therapeutic doses and to the fact that mammals do not have glutamate-gated chloride channels (the specific site of action for the MLs in target parasites). Additionally, these drugs do not cross the blood–brain barrier. The presence of P-GP in the blood–brain barrier, as well as in other tissues, serves as an important protective biological barrier to any adverse effects of the MLs.

Ecotoxicological Impact

The evaluation of the pattern of fecal elimination of different ML compounds administered by different routes to cattle have received special attention, due to the potential negative environmental impact caused by the effect of the drugs against dung-degrading insects, which has been evaluated under different conditions, initially for ivermectin (Lumaret et al., 1993; Herd, 1995) and then for other MLs (Suarez et al., 2003). The prolonged presence of these molecules in the feces of treated cattle produces an adverse effect against invertebrate organisms that have a relevant role on dung degradation and nutrients recycling to the soil (Strong, 1993). The residues of ivermectin and abamectin in feces not only affect the development and reproduction of adults *Coleoptera* but also are toxic against larval stages between 2 and 4 weeks after an injectable treatment (Strong and Wall, 1994; Herd, 1995). The consequences of this toxicity over the ecosystem are not fully understood. Moxidectin has been considered to be a less harmful compound compared to the avermectin-type compounds following standard injectable treatments. The lower fecal concentrations measured after moxidectin treatment in cattle compared to those obtained after ivermectin and doramectin administration (Lifschitz et al., 1999a), with a possible higher rate of environmental degradation of moxidectin, may determine its lower toxicity against dung fauna. Further research is required to evaluate potential adverse environmental impact of the ML compounds under a variety of conditions. Long-term investigations under different field production systems are particularly needed.

Concluding Remarks

The discovery of avermectins and the commercial launch of ivermectin as an ecto-endoparasiticidal agent in the early 1980s were great breakthroughs within veterinary therapeutics, which was successfully followed by the development of other avermectin and milbemycin compounds. The MLs from both chemical families combine excellent spectrum with safety, potency, and persistence. They are highly efficacious antiparasitic agents for use in a variety of livestock and companion animal species. The observations highlighted in this chapter on the phamacokinetic/pharmacodynamic relationship for MLs provide the pharmacological knowledge required to improve their therapeutic use. Avoiding misuse of the MLs is critical to delay the development of resistance. The integrated assessment of the drug disposition kinetics in the host, the processes of drug diffusion into different target parasites, and the use of pharmaceutical technology to improve drug delivery at the site of infection, are key research areas within the pharmacology of the antiparasitic drugs.

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42

Antiprotozoan Drugs

Jennifer L. Davis and Jody L. Gookin

Numerous chemotherapeutic agents are known to possess activity against parasitic protozoa (Tables 42.1 and 42.2). Although antiprotozoal activity is characteristic of many different chemical groups, most possess a rather narrow spectrum of activity. This is in contrast to antibacterial agents, which are effective against a wide variety of organisms. This chapter presents the clinically relevant antiprotozoan drugs used against the more important protozoal disease agents of domesticated animals.

Nitroimidazoles

Spectrum: *Giardia lamblia, Balantidium coli, Entamoeba histolytica, Tritrichomonas foetus, Pentatrichomonas hominis, Trichomonas gallinae, Histomonas maleagridis, Trypanosomas spp.*

Drugs included: metronidazole, tinidazole, ronidazole, dimetridazole, ornidazole, carnidazole, benznidazole, ipronidazole, and secnidazole (Figure 42.1).

Many of these important agents were used in the treatment of poultry flagellates, and all except metronidazole and tinidazole have been removed from the market in the United States. Nitroimidazoles are suspected mutagens and carcinogens; therefore their use in food-producing animals has been strictly prohibited by the US FDA. (Legal control of veterinary drugs is discussed in more detail in Chapter 55.) For companion, exotic, and zoo animals, metronidazole is the most commonly used agent in this group and the most studied.

It is convenient to think of the mode of action of nitroimidazoles as occurring in four successive steps (Finegold and Mathisen, 1990). First is entry into the protozoan cell, second is reductive activation, third is toxic effect of reduced intermediates, and fourth is release of inactive end products. The protozoal toxicity is due to short-lived intermediates or free radicals that produce damage by interacting with DNA and possibly other molecules. The cytotoxic intermediates decompose to nontoxic and inactive compounds.

Metronidazole

Metronidazole (Flagyl® and generic) is a weak base that is moderately lipophilic. It has a low molecular weight compared to other drugs (MW 171), which facilitates penetration across membranes. The oral absorption in most animals is almost complete and it reaches high concentrations in the tissues; therefore it is active against both luminal and extraluminal protozoa (Finch and Snyder, 1986). Bioavailability of metronidazole is good in most species. In the horse, the bioavailability ranges from 75 to 116% and is not affected by feed (Britzi et al., 2010; Steinman et al., 2000; Sweeney et al., 1986). Similarly, bioavailability is complete in foals; however, pharmacokinetics are age dependent, with reduced clearance noted in younger animals (Swain et al., 2015). In the dog, it is reported to be 60–100% (Neff-Davis et al., 1981). It has a half-life of about 6–8 hours, and less than 20% binds to plasma proteins. It is metabolized in the liver by oxidation and glucuronide formation and is excreted primarily by the kidneys. Small amounts may be found in saliva and breast milk (Finch and Snyder, 1986).

Metronidazole is usually well tolerated, but potential adverse reactions include glossitis, stomatitis, and nausea. The most important adverse effects are vomiting, neurotoxicosis, and neurotoxicity (Longhofer, 1988). The reactions observed appear to be caused by inhibition of the γ-aminobutyric acid (GABA) neurotransmitter. At high doses (67–129 mg/kg/day) metronidazole has caused ataxia, lethargy, proprioceptive deficits, nystagmus, and seizure-like signs in dogs. Dogs have recovered if drug administration was discontinued, but it may require 1 to 2 weeks (Dow, 1988; Dow et al., 1989). However, when diazepam was administered as a treatment, recovery was much faster (Evans et al., 2003). Neurotoxicosis also has been observed in cats with high doses. Metronidazole has been shown in cats to induce DNA damage in circulating peripheral blood mononuclear cells both in vitro and in vivo in as few as 7 days of treatment. This DNA damage appears reversible as it is no longer observed 7 days after drug

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Table 42.1 Summary of drugs used to treat selected protozoal diseases in animals

Protozoal disease	Protozoa	Species	Drug class	Drug and dosing regimen
Giardiasis	*Giardia duodenalis, G. intestinalis, G. lambia*	Dogs	Nitroimidazoles	Metronidazole: 12–15 mg/kg PO q12h for 8 days Tinidazole: 15 mg/kg PO q12h
			Benzimidazoles	Albendazole: 25 mg/kg q12h for 2 days Fenbendazole: 50 mg/kg q24h for 3 days Febantel, pyrantel, and praziquantal: febantel (26.8–35.2 mg/kg), pyrantel (26.8–35.2 mg/kg), praziquantel (5.4–7 mg/kg) PO q24h for 1–3 days
		Cats	Nitroimidazoles	Metronidazole: 22–25 mg/kg PO q12h for 5–7 days Tinidazole: 15 mg/kg PO q24h Secnidazole: 30 mg/kg PO q24h
			Benzimidazoles	Fenbendazole: 50 mg/kg q24h for 5 days Febantel, pyrantel, and praziquantal: febantel (56.5 mg/kg), pyrantel (11.3 mg/kg), and praziquantel (37.8 mg/kg) PO q24h for 5 days
		Horses	Nitroimidazoles	Metronidazole: 5 mg/kg PO q8h for 10 days
		Cattle	Benzimidazoles	Albendazole: 20 mg/kg PO q24h for 3 days Fenbendazole: 5–20 mg/kg PO q24h for 3 days
			Aminoglycosides	Paromomycin: 75 mg/kg PO q24h for 5 days
Trichomoniasis	*Tritrichomonas foetus, Pentatrichomonas hominis, Trichomonas gallinae*	Cats	Nitroimidazoles	Tinidazole: 30 mg/kg PO q24h for 14 days Ronidazole: 30 mg/kg PO q24h for 14 days
		Cattle	Nitroimidazoles	Metronidazole: 75 mg/kg IV q12h for 3 doses[a]
		Pigeons	Nitroimidazoles	Metronidazole: 40–60 mg/kg orally once a day for 5 days Ronidazole: 5 mg/kg orally once a day for 14 days Carnidazole: 20 mg/kg orally once
Trypanosomiasis	*Trypanosomas cruzi*	Dogs	Nitroimidazoles	Benznidazole: 5–7 mg/kg PO q24h for 2 months
			Nitrofurans	Nifurtimox: 2–7 mg/kg PO q6h for 3–5 months
		Cattle	Diamidene derivatives	Diminazene diaceturate: 3.5–7 mg/kg IM
Babesiosis	*Babesia divergens,*	Dogs	Tetracyclines	Doxycycline: 10 mg/kg PO q12h for 11 days (*B. canis*) Doxycycline (7–10 mg/kg, q12h) in combination with enrofloxacin (2–2.5 mg/kg, q12h) and metronidazole (5–15 mg/kg, q12h) for a duration of 6 weeks (*B. gibsoni*)
	B. equi, B. caballi, B. bigemini, B. canis		Hydroxyquinolones and naphthoquinones	Atovaquone: 13.3 mg/kg PO q8h combine with azithromycin 10 mg/kg PO q24h for 10 consecutive days (*B. gibsoni*)
			Diamidene derivatives	Diminazene diaceturate: 3.5–5 mg/kg BW IM (*B. canis*); 3.5 mg/kg q12h for 2 treatments (*B. gibsoni*) Pentamidine isethionate: 16.5 mg/kg IM on 2 consecutive days Phenamidine isethionate: 15–20 mg/kg SC q24h for 2 days (*B. gibsoni*); 8–13 mg/kg SC once (*B. canis*) Imidocarb dipropionate: 7.5 mg/kg SC (*B. canis*); 3.5 mg/kg diminazene followed by 6 mg/kg imidocarb on the following day (*B. canis*)
		Horses	Tetracyclines	Chlortetracycline: 0.5–2.6 mg/kg IV q24h for 6 days (*B. equi*)
			Diamidene derivatives	Diminazene diaceturate: 3–5 mg/kg IM q12h for 2 treatments (*B. caballi*); 6–12 mg/kg IM (*B. equi*); 0.5 mg/kg IM (*B. bigemina*) Phenamidine isethionate: 8.8 mg/kg IM q12h for 2 treatments Amicarbalide: 8.8 mg/kg IM q12h for 2 treatments (*B. caballi*) Imidocarb dipropionate: 1–2 mg/kg IM q12h for 2 treatments (*B. caballi*); 4 mg/kg IM q72h for 4 treatments (*B. equi*)
		Cattle	Tetracyclines	Oxytetracycline: 20 mg/kg IM every 4 days for 3 weeks (*B. divergens*)
			Diamidene derivatives	Diminazene diaceturate: 3–5 mg/kg IM (*B. bigemina* and *B. bovis*) Pentamidine isethionate: 0.5–2 mg/kg SC Phenamidine isethionate: 8–13 mg/kg IM q12h for 2 treatments Amicarbalide: 5–10 mg/kg IM Imidocarb dipropionate: 1–3 mg/kg IM or SC

(*continued*)

Table 42.1 (*Continued*)

Protozoal disease	Protozoa	Species	Drug class	Drug and dosing regimen
Cryptosporidiosis	*Cryptosporidium parvum*	Cats	Aminoglycosides	Paromomycin: 165 mg/kg PO q12h for 5 days[b]
			Nitrothiazole derivatives	Nitazoxanide: 75 mg/kg PO once
		Cattle	Aminoglycosides	Paromomycin: 12.5–50 mg/kg PO q24h for 12 days
			Azalides	Azithromycin: 1500–2000 mg/calf/day PO for 7 days
			Nitrothiazole derivatives	Nitazoxanide: 1.5 g suspended in water and given orally q12h
			Alkaloids	Halofuginone: 100 μg/kg BW PO q24h for 7 days
		Goats	Aminoglycosides	Paromomycin: 50 mg/kg PO q12h for 10 days
Leishmaniasis	*Leishmania donovani, L. infantum, L. chagasi*	Dogs	Pentavalent antimonials	Sodium stibogluconate: 30–50 mg/kg pentavalent antimony IV or SC q24h for 3–4 weeks Meglumine antimonate: 100 mg/kg IV or SC q24h for 3–6 weeks or 75 mg/kg SC q12h in combination with allopurinol at 15 mg/kg PO q12h Liposomal meglumine antimonate: 9.8 mg/kg IM or SC q24h
Hepatozoonosis	*Hepatozoon americanum*	Dogs	Aminoglycosides	Paromomycin: 20–40 mg/kg IM q24h for 15–30 days
			Hydroxyquinolones and naphthoquinones	Decoquinate: feed additive (27.2 grams/pound or 59.8 grams/kg of premix) added to moist dog food at 0.5–1.0 tablespoons per 10 kg BW twice a day
			Triazene derivatives	Toltrazuril: 5–10 mg/kg PO q12h for 5–10 days
			DHFR/TS[c] inhibitors	Trimethoprim–sulfadiazine: 15 mg/kg PO q12h combined with clindamycin 10 mg/kg PO q8h and pyrimethamine 0.25 mg/kg PO q12h for 14 days[d]
			Diamidene derivatives	Imidocarb dipropionate: 5–6 mg/kg BW SQ or IM every 2 weeks until gamonts are no longer present in blood smears
Cytauxzoonosis	Cytauxzoon felis	Cats	Diamidene derivatives	Imidocarb dipropionate: 3.5 mg/kg BW IM once then repeat 7 days later
			Hydroxyquinolones and naphthoquinones	Atovaquone: 15 mg/kg PO q8h combine with azithromycin 10 mg/kg PO q24h for 10 consecutive days
Theileriosis	*Theileria parva*	Cattle	Hydroxyquinolones and naphthoquinones	Parvaquone: 20 mg/kg IM once Buparvaquone: 2.5 mg/kg IM 1–2 times
			Alkaloids	Halofuginone: 1–2 mg/kg PO once
Equine Protozoal Myelitis	*Sarcocystis neurona*	Horses	Triazene derivatives	Diclazuril: 0.5–1 mg/kg PO q24h for 28 days, or 0.5 mg/kg PO, once every 3–4 days. Toltrazuril: 5 mg/kg PO q24h for 28 days Ponazuril: 15 mg/kg PO loading dose; 5 mg/kg PO q24h for 28 days
			DHFR/TS inhibitors	Pyrimethamine: 1 mg/kg PO q24h combined with sulfadiazine 20–30 mg/kg PO q12h for 30 days after clinical signs plateau
			Nitrothiazole derivatives	Nitazoxanide: 11.36 mg/lb (25 mg/kg) PO q24h for the first 5 days, followed by 22.72 mg/lb PO q24h for 23 days
			Hydroxyquinolones and naphthoquinones	Decoquinate: 0.5 mg/kg PO q24h combined with levamisole 1 mg/kg PO q24h for 10 days
Neosporosis	*Neospora caninum*	Dogs	DHFR/TS inhibitors	Pyrimethamine: 0.25–0.5 mg/kg PO q12h combined with sulfadiazine 30 mg/kg PO q12h for 2–4 weeks
			Lincosamides	Clindamycin: 12.5–18.5 mg/kg PO q12h for 2–4 weeks
		Cattle	Triazene derivatives	Ponazuril: 20 mg/kg PO q24h for 6 days
Toxoplasmosis	*Toxoplasma gondii*	Dogs	DHFR/TS inhibitors	Pyrimethamine: 0.25–0.5 mg/kg PO q12h combined with sulfadiazine 30 mg/kg PO q12h for 2–4 weeks
			Lincosamides	Clindamycin: 3–13 mg/kg PO or IM q8h for 2 weeks or 10–20 mg/kg PO or IM q12h for 2 weeks
		Cats	DHFR/TS inhibitors	Pyrimethamine: 0.25–0.5 mg/kg PO q12h combined with sulfadiazine 30 mg/kg PO q12h for 2–4 weeks
			Lincosamides	Clindamycin: 10–12 mg/kg PO q12h for 4 weeks or 12.5–25 mg/kg IM q12h for 4 weeks

Table 42.1 (*Continued*)

Protozoal disease	Protozoa	Species	Drug class	Drug and dosing regimen
Sarcocystis	*Sarcocystis spp.*	Dogs	Hydroxyquinolones and naphthoquinones	Decoquinate: 10–20 mg/kg PO q12h
Coccidiosis	*Eimeria sp., Isospora sp.*	Dogs	DHFR/TS inhibitors	Ormetoprim/sulfadimethoxine: 11 mg/kg ormetoprim combined with 55 mg/kg sulfadimethoxine PO for up to 23 days Trimethoprim/sulfadiazine: 5–10 mg/kg trimethoprim combined with 25–50 mg/kg sulfadiazine PO q24h for 6 days to dogs over 4 kg; dogs under 4 kg BW are given half this dosage for 6 days
			Thiamidine derivatives	Amprolium: 100–200 mg total PO q24h for 7–12 days; in drinking water (sole source) at 30 ml (9.6% solution)/gal for up to 10 days; in food at 250–300 mg (20% powder) q24h for 7–10 days
			Sulfonamides	Sulfadimethoxine: 55 mg/kg PO q24h for 1 day, then 27.5 mg/kg PO q24h for 14–20 days
			Triazine derivatives	Toltrazuril: 9 mg/kg BW PO once Ponazuril: 50 mg/kg BW PO q24h for 3 days
		Cats	Sulfonamides	Sulfadimethoxine: 55 mg/kg PO q24h for 1 day, then 27.5 mg/kg PO q24h for 14–20 days
			DHFR/TS inhibitors	Trimethoprim/sulfadiazine: 5–10 mg/kg trimethoprim combined with 25–50 mg/kg sulfadiazine PO q24h for 6 days to cats over 4 kg; cats under 4 kg BW are given half this dosage for 6 days.
			Thiamidine derivatives	Amprolium: 110–220 mg/kg PO q24h for 7–12 days; in drinking water (sole source) at 1.5 tsp (9.6% solution)/gal for up to 10 days; 150 mg/kg PO combined with sulfadimethoxine at 25 mg/kg PO q24h for 14 days.
			Triazine derivatives	Toltrazuril: 18 mg/kg BW PO once Ponazuril: 50 mg/kg BW PO q24h for 3 days

[a] The use of nitroimidazoles in food-producing animals is strictly prohibited by the US FDA. Cattle receiving metronidazole should never enter the food chain.

[b] See text for discussion of toxicity.

[c] Dihydrofolate reductase/thyamidine synthase inhibitors.

[d] Regimen should be followed up with decoquinate therapy.

withdrawal (Sekis et al., 2009). Histological evidence of peripheral neurotoxicity and hepatotoxicity were noted following long term (30 days) treatment in horses treated at doses of 30 mg/kg q 12 h orally (White et al., 1996).

Metronidazole is notoriously unpalatable. This may create difficulty for administering to difficult-to-medicate pets (cats), may be challenging to administer to horses, and the aftertaste may be accompanied by a loss of appetite.

Metronidazole Benzoate

In an attempt to provide a more palatable formulation, compounding pharmacists have provided metronidazole as an ester of benzoic acid-metronidazole benzoate, also known as *benzoylmetronidazole*. This formulation, although not approved by the FDA, is available from compounding pharmacists and has been popular for use in cats. The oral absorption is 65% and it has a half-life in cats of approximately 5 hours (Sekis et al., 2009). Metronidazole base is soluble (10 mg/ml), and has moderate lipophilicity (logP −0.02 and log D −0.27 at pH 5 and above). By contrast, the benzoate form, which is an ester, is much less soluble but more lipophilic (log P value of 2.19; log D value of 2.19 at pH 5.0 and above). The lower solubility results in less drug dissolved in the saliva of cats and therefore does not produce the degree of unpleasant taste compared to the base. The lipophilicity allows for good oral absorption from the GI tract. At a dose of 20 mg/kg of metronidazole benzoate, which is equivalent to 12.4 mg/kg of metronidazole, concentrations following the oral dose in cats were above the MIC$_{90}$ for most anaerobic bacteria for at least 12 hours and above 1 μg/ml (for more susceptible organisms) for at least 24 hours (Sekis et al., 2009). Although cats are sensitive to benzoic acid toxicity, at these dosages it is unlikely that administration of metronidazole benzoate will exceed a level to produce toxicosis in cats.

Table 42.2 Drugs licensed for the treatment of coccidiosis in food animals

Species	Drug	Trade name	Regulatory information
Chickens	Roxarsone	Ren-O-Sal®, Roxarsone 10% Type A Medicated Article, combination products available	Not for use in laying hens; 5-day slaughter withdrawal for noncombination products, no longer marketed
	Decoquinate	Deccox® Type A Medicated Article, combination products available	Not for use in laying hens; no withdrawal necessary for noncombination products
	Clopidol	Cloyden 25®, combination products available	Not for use in laying hens; 5-day slaughter withdrawal for noncombination products
	Robenidine	Robenz®, combination products available	Not for use in laying hens; 5-day slaughter withdrawal for noncombination products
	Amprolium	Amprol 25%, CORID 25% Type A Medicated Article, combination products available	No withdrawal necessary for noncombination products
	Dinitolmide	Zoamix®	Not for use in laying hens; no withdrawal necessary
	Nicarbazin	Nicarbazin, combination products available	Not for use in laying hens; 4-day slaughter withdrawal for noncombination products
	Halofuginone	Stenerol®, combination products available	Not for use in laying hens; 4-day slaughter withdrawal for noncombination products
	Lasalocid	Avatec®, combination products available	Not for use in laying hens; no withdrawal necessary for noncombination products
	Maduramicin	Cygro®	Not for use in laying hens; 5-day slaughter withdrawal for noncombination products
	Monensin	Coban®, combination products available	Not for use in laying hens; no withdrawal necessary
	Narasin	Monteban®, combination products available	Not for use in laying hens; no withdrawal necessary
	Semduramicin	Aviax®, combination products available	Not for use in laying hens; no withdrawal necessary for noncombination products
	Salinomycin	Bio–Cox®, Sacox®	Not for use in laying hens; no withdrawal necessary
	Diclazuril	Clinacox®, combination products available	Not for use in laying hens; no withdrawal necessary for noncombination products
	Ormetoprim/ sulfadimethoxine	Rofenaid®, combination products available	Not for use in laying hens; 5-day slaughter withdrawal for noncombination products
	Sulfamethazine	Sulmet® Drinking Water Solution	Not for use in laying hens; 10-day slaughter withdrawal for noncombination products
Turkeys	Roxarsone	Ren-O-Sal®, Roxarsone 10% Type A Medicated Article, combination products available	Not for use in laying animals; 5-day slaughter withdrawal for most products, no longer marketed
	Clopidol	Cloyden 25®, combination products available	Not for use in laying animals; 5-day slaughter withdrawal for noncombination products
	Amprolium	Amprol 25%, CORID 25% Type A Medicated Article, combination products available	No withdrawal necessary for noncombination products
	Dinitolmide	Zoamix®	Not for use in laying animals; no withdrawal necessary
	Halofuginone	Stenerol®, combination products available	Not for use in laying animals; 7-day slaughter withdrawal for noncombination products
	Lasalocid	Avatec®, combination products available	Not for use in laying animals; no withdrawal necessary for noncombination products
	Monensin	Coban®, combination products available	Not for use in laying animals; no withdrawal necessary for noncombination products
	Diclazuril	Clinacox®, combination products available	Not for use in laying hens; no withdrawal necessary
	Ormetoprim/ sulfadimethoxine	Rofenaid®, combination products available	Not for use in laying animals; 5-day slaughter withdrawal for noncombination products
	Sulfamethazine	Sulmet® Drinking Water Solution	Not for use in laying hens; 10-day slaughter withdrawal for noncombination products

Table 42.2 (*Continued*)

Species	Drug	Trade name	Regulatory information
Cattle	Decoquinate	Deccox® Type A Medicated Article, combination products available	Not for use in lactating dairy cattle; approved for preruminating calves; no withdrawal necessary for noncombination products
	Amprolium	Amprol 25%, CORID 25% Type A Medicated Article, combination products available	Not for use in preruminating calves; 1-day slaughter withdrawal
	Sulfamethazine	Sulmet® Oblets, Sulfamethazine sustained release bolus	Not for use in lactating dairy cattle; 8 to 10-day slaughter withdrawal for individual products
	Sulfaquinoxaline	Sulfa-nox Liquid	Not for use in lactating dairy cattle; 10-day slaughter withdrawal for noncombination products
Sheep	Decoquinate	Deccox® Type A Medicated Article, combination products available	Not for use in lactating sheep; no withdrawal necessary for noncombination products
	Lasalocid	Bovatec®, numerous others and combination products available	Not for use in breeding animals; no withdrawal necessary for noncombination products
Goats	Decoquinate	Deccox® Type A Medicated Article, combination products available	Not for use in lactating goats; no withdrawal necessary for noncombination products
	Monensin	Rumensin®	Not for use in lactating goats; no withdrawal necessary
Swine	Toltrazuril	Baycox®	Extralabel drug use, contact regulatory agencies for withdrawal times
Rabbits	Diclazuril	Clinicox®	Extralabel drug use, contact regulatory agencies for withdrawal times
	Lasalocid	Avatec/Bovatec®, numerous others and combination products available	No withdrawal necessary for noncombination products
	Sulfaquinoxaline	SQ 40% Medicated feed	10-day withdrawal
Pheasants	Amprolium	Amprol 25%, CORID 25% Type A Medicated Article, numerous others and combination products available	No withdrawal necessary for noncombination products
Chukar partridges	Lasalocid	Avatec/Bovatec®	No withdrawal up to 8 weeks of age
	Ormetoprim/ sulfadimethoxine	Rofenaid®	Not released into wild before 18 weeks and can be used only up to 8 weeks of age
Bobwhite quail	Monensin	Coban®, combination products available	Not for use in laying hens; no withdrawal necessary
	Salinomycin	Bio-Cox®, Sacox®	No withdrawal necessary
Ducks	Ormetoprim/ sulfadimethoxine	Rofenaid®	Not for use in breeding animals or animals producing eggs for human consumption; 5-day slaughter withdrawal

Please see text for further explanation of dosing and toxicities. Regulatory information is applicable to drugs used in the United States, and is current only as of the writing of this chapter. Please consult the label directions on all products prior to administration because extralabel use of feed additives is prohibited by the US FDA.

Clinical Use

Metronidazole is not approved for animal use, but is available as the base in capsules (375 mg), tablets (250 and 500 mg), or for injection as metronidazole hydrochloride in 500 mg vials. In addition to the antiprotozoal uses listed here, the major use of metronidazole in animals is for the treatment of infections caused by anaerobic bacteria. Metronidazole has a unique effect on anaerobic bacteria because of a reduction reaction to cytotoxic derivatives, a mechanism lacking in aerobic bacteria. Discussion on the antibacterial uses of metronidazole is listed in Chapter 36.

Cats: Metronidazole at a dose of 25 mg/kg orally, twice daily for 7 days has been shown to completely eliminate *Giardia* cyst shedding in cats known to be chronic shedders of a human isolate of *Giardia lamblia* (Scorza and Lappin, 2004). Metronidazole was also effective in eliminating cyst shedding as well as diarrhea associated with naturally occurring *Giardia* infections at a dose of 22 mg/kg orally, twice daily for 5 days (Zimmer, 1987).

Dogs: Metronidazole at a dose of 12–15 mg/kg orally, twice a day for 8 days is commonly prescribed for treatment of infections caused by *Giardia lamblia*, *Balantidium coli*, *Entamoeba* spp., and *P. hominis*.

Horses: Giardia infections in horses are rarely reported to cause disease; however, successful treatment has been accomplished with 5 mg/kg of metronidazole orally, three times a day for 10 days (Kirkpatrick and Skand, 1985).

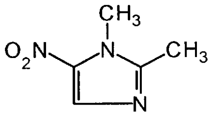
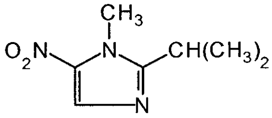
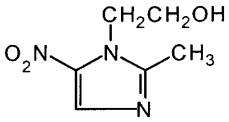
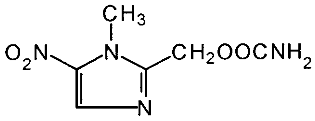
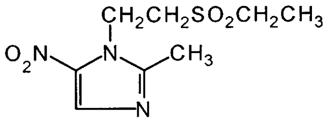
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Dimetridazole	1,2-dimethyl-5-nitro-1*H*-imidazole ($C_5H_7N_3O_2$) [141.13]	
Ipronidazole	1-methyl-2-(1-methylethyl)-5-nitro-1*H*-imidazole ($C_7H_{11}N_3O_2$) [169.18]	
Metronidazole	1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole ($C_6H_9N_3O_3$) [171.16]	
Ronidazole	1-methyl-2-[(carbamoyloxy)methyl]-5-nitroimidazole ($C_6H_8N_4O_4$) [200.16]	
Tinidazole	1-[2-(ethylsufonyl)ethyl]-2-methyl-5-nitro-1*H*-imidazole ($C_8H_{13}N_3O_4S$) [247.26]	

Figure 42.1 Nitroimidazoles.

Cattle: Metronidazole has been used to treat trichomoniasis in bulls at a dose of 75 mg/kg IV q 12 h for up to three doses. Metronidazole (and related drugs) are strictly forbidden for use in food-producing animals. Because of regulatory issues, animals treated in this manner must never enter the food chain in the United States.

Reptiles: Metronidazole is commonly used in reptile medicine for treatment of anaerobic bacteria and protozoa infections. However, there is little guidance on dosage regimens and studies of clinical effectiveness. Generally, doses of 20 mg/kg are administered. A summary of available studies is found in the paper by Innis et al. (2007). In red-eared slider turtles the half-life was 27 hours and it reached therapeutic concentrations at a dose of 20 mg/kg (Innis et al., 2007). However, metronidazole was administered by an intracoelomic injection, which produced adverse effects in some of the turtles.

Tinidazole

Tinidazole (Tindamax^{TM}, Simplotan®, or Fasigyn®) is a second-generation 5-nitroimidazole that is FDA approved for use in treating *T. vaginalis*, *Giardia* spp., and *Entamoeba histolytica* infections in people. Tinidazole is completely absorbed after oral administration in horses, cats, and dogs (Pyörälä et al., 1990; Sarkiala et al., 1991), and has been shown to penetrate well into various tissues where drug concentrations are found to be similar to that in plasma (Wood et al., 1973). The apparent total plasma clearance of the drug is about twofold higher in dogs than in cats, resulting in an elimination half-life that is twice as long in cats (8.4 hours) than in dogs (4.4 hours) (Sarkiala et al., 1991). The elimination half-life in horses is reported as 5.2 hours (Pyörälä et al., 1990). It is metabolized in the liver by hydroxylation and glucuronide conjugation and is excreted into both urine and feces. Tinidazole has been administered to dogs at doses up to 150 mg/kg body weight for 24 weeks without toxic or adverse reactions. Dosages of 450 mg/kg body weight may result in liver enzyme elevations in dogs. Toxicity data in cats has not been reported.

Clinical Use

Dogs and cats: Tinidazole at a dose of 30 mg/kg orally once a day for 14 days has been shown to suppress shedding of *T. foetus* in experimentally infected cats (Gookin et al., 2007). Based on studies in people, tinidazole is also likely to be effective for treatment of most infections amenable to metronidazole, including *Giardia* infections in dogs and cats. Pharmacokinetic studies suggest that doses of 15 mg/kg orally twice a day for dogs or once a day for cats will produce concentrations at a level that is thought to be therapeutically effective.

Horses: Clinical use of tinidazole in horses has not been reported. Pharmacokinetic studies suggest a dose of 10–15 mg/kg orally twice a day (Pyörälä et al., 1990).

Ronidazole

Ronidazole is not approved for human or veterinary use in the United States and not available in a marketed form; however, it is considered an effective treatment for *T. gallinae, Histomonas meleagridis, Giardia*, and *T. foetus* infections. It has a tenfold higher in vivo activity against trichomonal infections compared to metronidazole (Miwa et al., 1986). The susceptibility of *Tritrichomonas foetus* has been reported in two studies (Kather et al., 2007; Gookin et al., 2006). In the study by Gookin et al. (2006) ronidazole was the most active compound tested for in vitro activity.

Clinical Use

Cats: Ronidazole at a dose of 30 mg/kg orally twice a day for 14 days has been shown to eliminate shedding of *T. foetus* in experimentally infected cats (Gookin et al., 2006). The pharmacokinetics of ronidazole has been examined in cats after IV and oral administration (LeVine et al., 2011). It was rapidly absorbed after oral administration in cats with a long terminal half-life. Although neurotoxicity has been reported from administration to cats (Rosado et al., 2007), these reactions were associated with high doses. Reports indicate that ronidazole treatment is discontinued because of signs of toxicity in approximately 5% of treated cats (Xenoulis et al., 2013). Based on the pharmacokinetic results cited above, current recommendations are to not exceed 30 mg/kg once daily in cats. In a pharmacokinetic study in which ronidazole was administered intravenously (prepared from an aqueous compounded formulation) and orally, no adverse reactions in healthy cats were observed. Resistance of *T. foetus* to treatment with ronidazole both in vivo and in vitro is increasingly recognized (Gookin et al., 2010).

Dogs: Ronidazole at a dose of 30–50 mg/kg orally twice a day for 7 days combined with environmental disinfection was reported to be effective in eliminating Giardia cyst and antigen shedding in a group of chronically infected kenneled dogs (Fiechter et al., 2012).

Pigeons: *T. gallinae* (pigeon canker) is generally treated with ronidazole (5 mg/kg orally once a day for 14 days), carnidazole (20 mg/kg orally once), or metronidazole (40–60 mg/kg orally once a day for 5 days).

Benznidazole

Benznidazole has been shown to produce cures of acute trypanosomiasis in dogs with fewer side effects than nifurtimox. Following experimental infection with *Trypanosoma cruzi*, benznidazole initially induced a potent suppression of parasitemia in treated animals. However, 12 months posttreatment, the parasite detection was similar between treated and untreated infected groups (Santos et al., 2012).

Clinical Use

Dogs: Dogs are treated with 5–7 mg/kg BW benznidazole orally at 24-hour or 12-hour intervals for 2 months (Barr, 2006; Santos et al., 2012).

Secnidazole

Secnidazole has been reported as effective for elimination of cyst shedding by cats experimentally infected with *Giardia* using a single dose given orally at 30 mg/kg of body weight. At this dose, however, cats were described as apathetic, inappetant for 48 hours after drug administration, and in a single cat an increase in measured liver enzyme activities were documented (Da Silva et al., 2011).

Pentavalent Antimonials

Spectrum: *Leishmania* spp.

Drugs included: Sodium stibogluconate, meglumine antimonate (Figure 42.2).

Pentavalent antimonials have been shown to inhibit topoisomerase in vitro (Lucumi et al., 1998) and thereby interfere with parasite replication. They also inhibit enzymes involved in the synthesis of nucleotides and inhibit phosphofructokinases, enzymes necessary for glycolytic and fatty acid oxidation (Baneth and Shaw, 2002). The in vivo mode of action of the pentavalent antimonials is still unclear. The pharmacodynamics of these drugs are still debated, and it is not known whether a better clinical response would be achieved with higher peak plasma concentrations versus prolonged time above the minimum inhibitory concentration of the protozoa (Valladares et al., 1998). Increasing resistance to pentavalent antimony compounds has been documented in recent years, and their effectiveness appears to be decreasing. In general, canine visceral leishmaniasis is more difficult

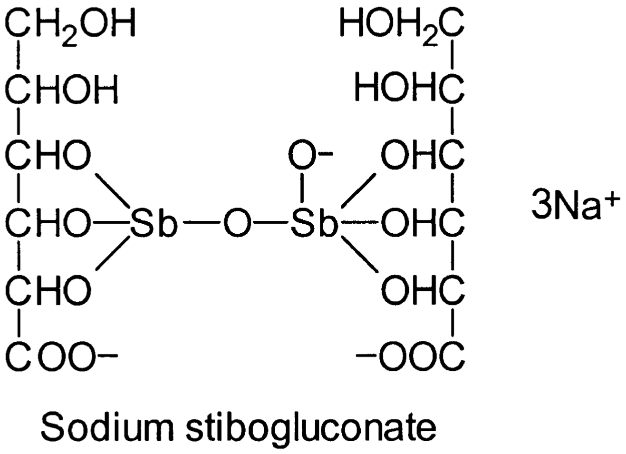


Figure 42.2

to treat than the human form of the disease. The use of pentavalent antimonials is contraindicated in patients with myocarditis, hepatitis, or nephritis. Both sodium stibogluconate and meglumine antimonate are administered on the basis of their antimony content. Paromomycin may increase the concentration of antimony compounds, thereby increasing toxicity, therefore caution is advised with coadministration (Belloli et al., 1995).

Sodium Stibogluconate

Sodium Stibogluconate (Pentostam®) was made available in the United States, from the Centers for Disease Control in 1968, for treatment of leishmaniasis in human beings (Figure 42.2). It is in aqueous solution at a concentration of 330 mg/ml of agent, which is equivalent to 100 mg/ml pentavalent antimony. Clinical formulations consist of multiple uncharacterized molecular forms, some of which have higher molecular weights than the active compound. In human beings, most of a single dose of sodium stibogluconate is excreted by the kidneys in the urine within 24 hours regardless of whether it is given IV or IM. Antimony compounds are eliminated faster if given IM than SC or IV in dogs, and it is important to maintain serum concentrations of the compound to treat leishmaniasis. Pentavalent antimonials are relatively well tolerated. Adverse reactions include pain at the injection site, gastrointestinal (GI) clinical signs, delayed muscle pain, and joint stiffness.

Clinical Use

Dogs: Canine leishmaniasis is treated with sodium stibogluconate to deliver 30–50 mg/kg BW pentavalent antimony by either IV or SC administration at daily intervals for 3–4 weeks (Slappendel and Teske, 1997). Relapses may occur a few months to a year after treatment and should be treated with another round of pentavalent antimony.

Meglumine Antimonate

Meglumine antimonate (Glucantime®) may be less likely to cause adverse effects than sodium stibogluconate. Adverse effects noted include lethargy, gastrointestinal disturbance, and injection site reactions, including muscle fibrosis and abscess formation with IM injection (Noli and Auxilia, 2005). It is available in a solution that contains 300 mg/ml antimony. Bioavailability is approximately 92% after IM or SC administration, and 80% of the drug is excreted in the urine within the first 9 hours (Valladares et al., 1997).

Liposomal encapsulated formulations of meglumine antimonite (LMA) have also been tested in dogs. They reach higher plasma concentrations, have slower clearance, and have a higher volume of distribution than regular formulations (Valladares et al., 1997). They also reach good concentrations in the bone marrow of affected dogs. Compared to conventional formulations, a positive effect on plasma total protein and gammaglobulin concentrations was seen with the LMA formulation, with no relapses noted at 12 months after treatment. Although good results have been seen with this formulation, complete eradication of the parasite is unlikely to be achieved and the product may not be cost effective when compared to conventional formulations.

Clinical Use

Dogs: Canine visceral leishmaniasis has been treated with meglumine antimonate given IV or SC at 100 mg/kg BW once daily for 3–6 weeks (Slappendel and Teske, 1997). No advantage is provided by IV administration. Relapses will occur in long-term survivors and require retreatment. Alternatively, a dosing regimen of 75 mg/kg SC twice daily achieves higher peak concentrations and maintains detectable plasma concentrations for the entire dosing interval (Valladares et al., 1998). For liposomal encapsulated meglumine antimonite, a dose of 9.8 mg/kg q 24 h is recommended (Valladares et al., 2001). Combined treatment with meglumine antimoniate and allopurinol is considered to be the most effective therapy for canine leishmaniasis (Manna et al., 2015). The combination is administered for 4 to 8 weeks, followed by treatment with allopurinol alone at a dose of 15 mg/kg orally twice a day for at least 6 to 12 months (Paradies et al., 2012). In an outbreak of cutaneous leishmaniasis in dogs in South America, empiric treatment with 14–16 mg/kg BW of meglumine antimoniate IM every day for up to 30 days resulted in good clinical cure in all dogs (n = 72), but relapses were reported 1–2 months after the end of treatment (Vélez et al., 2012).

Arsenicals

Spectrum: Histomoniasis, coccidiosis

Drugs included: Arsanilic acid, carbarsone, nitarsone, and roxarsone.

The exact antiprotozoal mechanism of action of arsenicals is not known, however these drugs nonspecifically bind to disulphide moieties in proteins, thereby inhibiting many enzyme systems that use biological ligands containing available sulfhydral groups (Blum and Burri, 2002). This mechanism is also thought to be related to the toxic effects of arsenic compounds on the host. These drugs continue to be administered to humans for treatment of African trypanosomiasis. In poultry, use of these compounds is for the prevention and treatment of histomoniasis and coccidiosis in turkeys.

Available veterinary formulations are restricted to organic arsenic compounds, as inorganic arsenic is considered carcinogenic. It has been found that organic arsenic can be converted to inorganic arsenic in vivo, resulting in detectable levels in the liver of poultry (Conklin et al., 2012). Although concentrations of inorganic arsenic are low in the tissue and unlikely to cause problems in the human food chain, this information has led to the systematic withdrawal of arsenical containing formulations from the market in the United States.

Clinical Use

Chickens and turkeys: Nitarsone (Histostat-50®) has been used as a feed additive for the prevention of histomoniasis in turkeys and chickens. It is not active if birds have been infected for more than 4 days. A 5-day withdrawal is required for nitarsone. Overdosing or lack of adequate water may result in leg weakness and paralysis in birds. Nitarsone is dangerous for ducks, geese, and dogs. Roxarsone (3-Nitro®) is fed as a growth promotant and as a preventative for coccidiosis. A 5-day withdrawal is also required for roxarsone. Like nitarsone, overdosing or lack of adequate water may result in leg weakness and paralysis in birds.

Benzimidazoles

Spectrum: Giardiasis

Drugs included: Albendazole, fenbendazole, febantel (Figure 42.3).

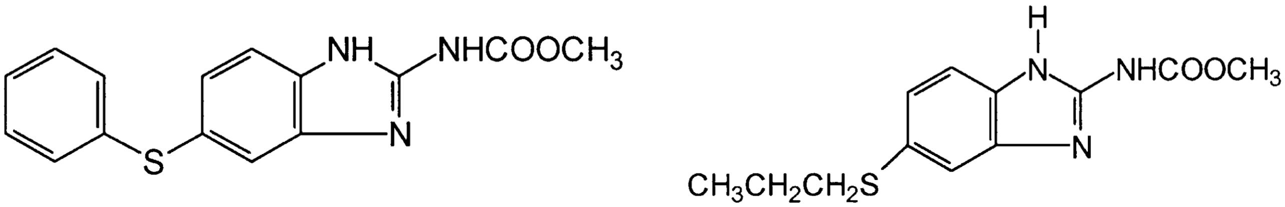
The benzimidazoles are a group of agents that are widely used in the treatment of helminth parasites of large and small animals. (Antihelmintics are discussed in detail in Chapters 39–41.) Some compounds in this group have excellent activity against *Giardia* spp. This group lacks, or has little, antibacterial activity and unlike other antigiardial agents is unlikely to interfere with intestinal microflora during treatment. The benzimidazoles are known to bind to β-tubulin subunits of microtubules and interfere with microtubule polymerization (Gardner and Hill, 2001). This causes structural changes in *Giardia* trophozoites consistent with microtubule damage to the adhesive disk and internal microtubule cytoskeleton but not the external flagella.

Albendazole

Albendazole (Valbazan®) is available in liquid and paste formulations. It is effective against *Giardia* sp. in human beings, mice, and dogs. Albendazole is poorly absorbed from the intestinal tract. Albendazole is potentially toxic, causing dose-dependent and idiosyncratic myelosuppression in dogs and cats respectively (Stokol et al., 1997). It is potentially teratogenic so it should not be given to pregnant animals. Its use in cats is not recommended.

Clinical Use

Dogs: A single oral 25 mg/kg BW treatment was not effective in clearing cysts from the feces of dogs, but dogs treated orally with albendazole at 25 mg/kg BW every



Fenbendazole
($C_{15}H_{13}N_3O_2S$)
[299.35]

Albendazole
($C_{12}H_{15}N_3O_2S$)
[265.33]

Figure 42.3

12 hours for 2 days cleared *Giardia* cysts from their feces (Barr et al., 1993). None of 32 dogs treated for giardiasis with albendazole developed adverse reactions (Barr et al., 1993).

Cattle: Albendazole given at 20 mg/kg BW orally for 3 days reduced *Giardia* cyst production by >90% in naturally infected calves (Xiao et al., 1996).

Fenbendazole

Fenbendazole (Panacur®, Safeguard®, Axilur®) is available in numerous preparations as a paste, suspension, or granules. Side effects with this drug are rare; however, vomiting and diarrhea may occur in small animals. Pancytopenia has been reported in a dog following fenbendazole administration (Gary et al., 2004). The changes were reversible with discontinuation of therapy.

Clinical Use

Dogs: Fenbendazole is effective against *Giardia* organisms in dogs if given at 50 mg/kg BW every 24 hours for 3 days (Zajac et al., 1998). More frequent dosing at 50 mg/kg BW orally every 8 hours was as effective as every 24 hours, and no adverse effects were noted (Barr et al., 1994a).

Cats: Fenbendazole at 50 mg/kg BW every 24 hours for 5 days was effective in eliminating the shedding of *Giardia* cysts in 4/8 cats coinfected with *Cryptosporidium* (Keith et al., 2003).

Cattle: Fenbendazole is also effective against *Giardia* organisms in calves when given orally at 5–20 mg/kg BW every 24 hours for 3 days (O'Handley et al., 1997; Xiao et al., 1996).

Febantel

Febantel (Drontal-Plus®, Drontal Flavour Plus®) is a benzimidazole available in a combination product with pyrantel pamoate or pyrantel embonate and praziquantel for the treatment of intestinal nematodes and cestodes in dogs and cats. Febantel is metabolized by the liver to fenbendazole and oxfendazole.

Clinical Use

Dogs: This combination has been shown to be effective to treat canine giardiasis when given orally, once daily for 1–3 days to provide praziquantel (5.4 to 7 mg/kg), pyrantel pamoate (26.8 to 35.2 mg/kg), and febantel (26.8 to 35.2 mg/kg) (Barr et al., 1998; Giangaspero et al., 2002). Strict hygiene to prevent reinfection is imperative to maximize treatment success (Payne et al., 2002). In studies of dogs naturally infected with *Giardia*, treatment with tablets containing 150 mg febantel, 144 mg pyrantel embonate, and 50 mg praziquantel at a dose of one tablet per 10 kg of body weight was effective in short-term eradication of cyst shedding (Bowman et al., 2009) and demonstrated no difference in efficacy if given orally once a day for a 3-day versus a 5-day duration (Montoya et al., 2008).

Cats: The combination product of febantel, pyrantel, and praziquantel was shown to be effective at decreasing *Giardia* cyst shedding in naturally infected kittens at doses of 56.5 mg/kg, 11.3 mg/kg, and 37.8 mg/kg, respectively, orally once a day for 5 days (Scorza et al., 2006). In addition, kittens treated at these doses were resistant to recrudescent shedding of oocysts following administration of methylprednisolone.

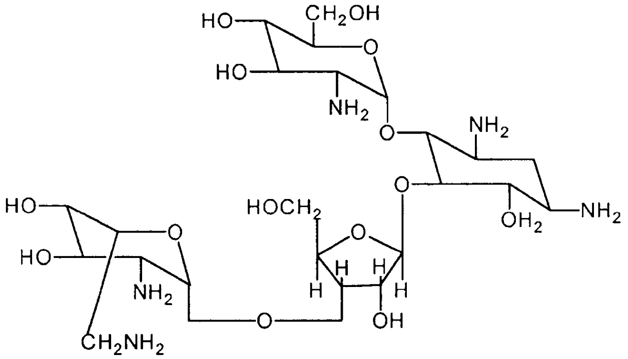
Aminoglycosides

Spectrum: *Giardia lamblia*, *Leishmania* spp., *Entamoeba* spp., *Cryptosporidium* spp.

Drugs included: Paromomycin (Figure 42.4).

Paromomycin

Paromomycin (Aminosidine®) is an aminoglycoside antibiotic that is produced by *Streptomyces rimosus*. Paromomycin interferes with bacterial protein synthesis by binding to 16S rRNA at the amino-acyl-tRNA binding site, which causes a conformational change and subsequent misreading of the mRNA and inhibition of translocation (Fourmy et al., 1998). Its anti-*Leishmania* mode



Paromomycin
($C_{23}H_{45}N_5O_{14}$)
[615.65]

Figure 42.4

of action is not known, but it has been suggested that it interferes with parasite mitochondrial activity (Maarouf et al., 1997).

Paromomycin is used in the treatment of luminal amoebiasis, leishmaniasis, and cryptosporidiosis. It has also been used in humans for the treatment of resistant giardiasis. It is poorly absorbed following oral administration, which leads to high intestinal luminal concentrations of the drug. Paromomycin has little activity against intestinal bacteria. Adverse effects include nausea, vomiting, abdominal cramps, and diarrhea. Although little is absorbed from the intestinal tract, it is eliminated via the kidneys, so its use is contraindicated in patients with renal disease. It is possible that in animals with intestinal disease, the mucosal barrier may be breached to produce more systemic absorption and potential for violative residues in food-producing species and systemic toxicity. This may have contributed to toxicity in four cats in one clinical study (Gookin et al., 1999). Coinfection with various mycotoxins, including fumonisin B1 and zearalenone, may also result in increased cellular permeability to paromomycin (Goossens et al., 2012). It also has the potential to cause reversible and irreversible vestibular, cochlear, and renal toxicity when given parenterally. Paromomycin plasma concentrations peak in the serum of dogs at 30 μg/ml about 60 minutes after IM or SC administration of a 15 mg/kg BW dose (Belloli et al., 1996). Paromomycin like other aminoglycosides has low protein binding in serum – approximately 4%.

Clinical Use

Dogs: Paromomycin given IM at 20 mg/kg BW daily for 15 days will greatly improve clinical signs of visceral leishmaniasis in dogs (Vexenat et al., 1998). Relapses may occur within 50–100 days. Administration of 15 mg/kg BW once daily, for 21 consecutive days resulted in a partial remission of the clinical signs and amelioration of clinicopathological abnormalities; however, a parasitological cure was not achieved (Athanasiou et al., 2013). Treatment with 40 mg/kg IM once daily for 30 days may enhance the cure rate of dogs (Vexenat et al., 1998). If paromomycin is administered SC with antimony, there is no effect on the pharmacokinetics of paromomycin but there is a marked effect on the pharmacokinetics of antimony (Belloli et al., 1995). Serum levels of antimony remain higher, and the dose should be adjusted to prevent toxic levels of the metal from appearing in the blood.

Cats: A naturally infected cat with cryptosporidial diarrhea was successfully treated with oral paromomycin given at 165 mg/kg BW orally every 12 hours for 5 days (Barr et al., 1994b). As mentioned previously, it can be toxic in cats if systemic absorption occurs owing to intestinal barrier disruption. It resulted in acute kidney disease, deafness, and hypermature cataract formation when given orally to four cats with *T. foetus* infection at doses as low as 70 mg/kg BW every 12 hours for 4 days (Gookin et al., 1999).

Cattle: For cryptosporidiosis, when administered at 12.5–25 mg/kg PO to calves, it delayed the onset of shedding and decreased the number of oocysts shed, while a dose of 50 mg/kg prevented oocyst shedding. Treatment of calves with oral paromomycin at 50, 25, or 12.5 mg/kg BW twice daily was effective in preventing oocyst production (50 mg/kg dose) or in delaying the onset of oocyst production and in greatly reducing the numbers of oocysts excreted (12.5–25 mg/kg BW doses) when started 1 day before inoculation and continued for 10 days after inoculation (Fayer and Ellis, 1993). For giardiasis, a dose of 75 mg/kg orally, once daily for 5 days caused a 100% reduction in cyst shedding at 9 days after initiation of treatment (Geurden et al., 2006).

Goats: Treatment of 2 to 4-day-old goat kids with oral paromomycin at 50 mg/kg BW twice daily for 10 days was effective in preventing cryptosporidiosis (Mancassola et al., 1995). Paromomycin may also have a role in treating or preventing the spread of cryptosporidiosis outbreaks in calves and goats. The anticryptosporidial activity of paromomycin apparently does not involve trafficking through the host cell cytoplasm but involves movement of the agent through altered apical membranes that surround the developing parasites (Griffiths et al., 1998). Paromomycin does not exert its effects on extracellular stages of *C. parvum* (Griffiths et al., 1998). There are no products containing paromomycin approved for use in food-producing animals in the United States; therefore, all use would be considered extralabel.

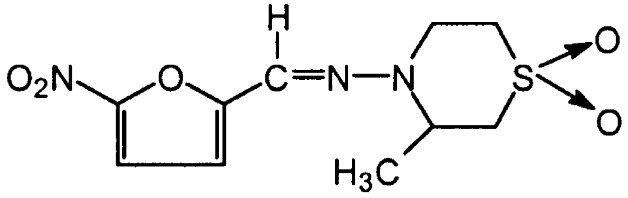
Nitrofurans

Spectrum: *Trypanosomas cruzi*

Drugs included: Nifurtimox (Figure 42.5).

Nifurtimox

Nifurtimox (Lampit®) is a nitrofuran derivative that is the most widely used agent for the treatment of human *Trypanosoma cruzi* infections (Van Reken and Pearson, 1990) and is effective against natural and experimental disease in dogs (Barr, 2006). It is marketed as 100 mg tablets, but in the United States must be obtained from the Centers for Disease Control. Mutagenicity and carcinogenicity of these compounds have been documented in laboratory animals; therefore, their use in



Nifurtimox
($C_{10}H_{13}N_3O_5S$)
[287.29]

Figure 42.5

food-producing animals, including the use of topical products, is strictly prohibited by the US FDA. (Legal control of veterinary drugs is discussed in Chapter 55.)

Its likely mode of action is through the production of activated forms of oxygen. It is reduced to the nitro anion radical in the presence of pyridine nucleotides. The anion then reacts with oxygen to produce superoxide and regeneration of nifurtimox (Finch and Snyder, 1986). This cycle continues, and the activated oxygen molecules exert their toxic effects on the parasites. This cycle is thought to be initiated by the enzyme trypanothione reductase. It has activity against amastigote and trypomastigote stages.

In human beings, nifurtimox is well absorbed after oral administration, but only low concentrations of the parent compound are found in the plasma and little is found in the tissues or urine (Webster, 1990). It is excreted in the urine in the form of metabolites. Adverse side effects occur in up to 50% of human beings treated and are associated with GI and central nervous system signs.

Clinical Use

Dogs: Canine *T. cruzi* infections are treated with 2–7 mg/kg BW nifurtimox orally at 6-hour intervals for 3–5 months (Barr, 2006). This is effective in preventing death from acute disease and extending life, but most dogs will still develop chronic cardiac disease, which is usually fatal (Barr, 2006). Improved survival has been shown in dogs concurrently treated with antiinflammatory doses of glucocorticoids (Andrade et al., 1980).

Tetracyclines

Spectrum: Amoebae, flagellates, piroplasms, ciliates.

Drugs included: Oxytetracycline, chlortetracycline, doxycycline, minocycline.

Tetracyclines represent a broad group of antiprotozoal agents, some of which have activity against amoebae, mucosal flagellates, piroplasms, and ciliates. (Chapter 34 has more detailed description of the pharmacology of tetracyclines.)

Clinical Use

Cattle: A long-acting formulation of oxytetracycline (LA/200) is useful in prophylaxis of bovine *Babesia divergens* infection if 20 mg/kg BW is given IM every 4 days for 3 weeks after exposure; 10–15 mg/kg BW given IM every 4 days causes moderation in the clinical signs (Kuttler, 1988). Tetracyclines can also be used to treat *Theileria* infections in cattle but are less effective and must be given in large doses early in the infection and used for longer periods of treatment. Product label for chlortracycline medicated feed includes control if active infection is caused by *Anaplasma marginale*. Oxytetracycline has been used in cattle for treatment of *Anaplasma marginale* infections. However, efficacy has been questionable. One study compared enrofloxacin (5 mg/kg IV q 24 h for 5 days), imidocarb (5 mg/kg IM twice, 7 days apart), or oxytetracycline (22 mg/kg IV q 24 h for 5 days) for elimination of persistent *Anaplasma marginale* infections in cattle. None of the tested treatments reliably eliminated persistent *A. marginale* infections in all cattle (Coetzee et al., 2006).

Horses: Chlortetracycline may be effective against *Babesia equi* infection in horses if 0.5–2.6 mg/kg BW is given IV daily for 6 days early in the infection (Kuttler, 1988). Oxytetracycline when administered at a dose of 5–6 mg/kg IV, once daily for 7 days was effective against *Theileria equi*, but not *Babesia caballi* (Zobba et al., 2008). Imidocarb (discussed in Section Imidocarb Dipropionate) is considered the most effective treatment for equine piroplasmosis (Wise et al., 2013).

Dogs: Doxycycline is effective at preventing clinical manifestations of *Babesia canis* infection if given at 10 mg/kg BW twice daily for 11 days (Vercammen et al., 1996). However, atovaquone in combination with azithromycin is considered the preferred treatment for *Babesia canis* in dogs (Birkenheuer, 2014). Treatment of dogs with *Babesia gibsoni* with a combination of oral doxycycline (7–10 mg/kg, q 12 h), enrofloxacin (2–2.5 mg/kg, q 12 h), and metronidazole (5–15 mg/kg, q 12 h) for a duration of 6 weeks, with and without two intramuscular injections of diminazene diaceturate (3 mg/kg, q 7 days), resulted in an overall efficacy of 85.7% and 83.3%, respectively, with a mean recovery time of 24.2 and 23.5 days, respectively (Lin et al., 2010). Combinations of clindamycin-doxycycline and metronidazole also have been used (Birkenheuer, 2014).

Hydroxyquinolones and Naphthoquinones

Spectrum: *Coccidia, Babesia* sp., *Hepatozoon americanum, Theileria* sp., *Cytauxzoon* sp., *T. gondii, Eimeria* sp., *malaria, Pneumocystis carnii.*

Drugs included: Decoquinate, atovaquone, parvaquone, buparvaquone (Figure 42.6).

The quinolone anticoccidials inhibit coccidial respiration by interfering with cytochrome-mediated electron transport in the parasites' mitochondria. The site of action of quinolone anticoccidials is probably within the bc1 complex, where the electrons are transferred from ubiquinone to cytochrome c. These compounds are coccidiostatic and allow penetration of sporozoites, but not development. These inhibited sporozoites have the ability to resume development after the agents are removed. Little anticoccidial immunity develops in chickens on these medications.

Decoquinate

Decoquinate (Deccox®) is one of the quinolone (4-hydroxyquinolones) anticoccidials. It is poorly absorbed

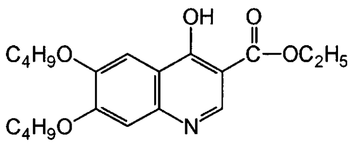
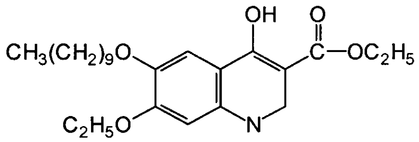
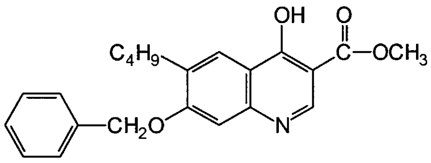
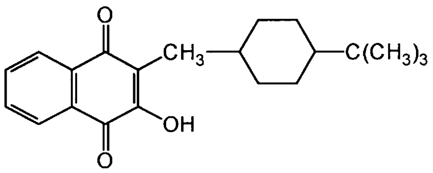
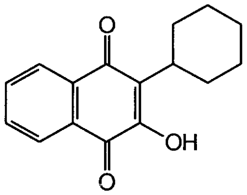
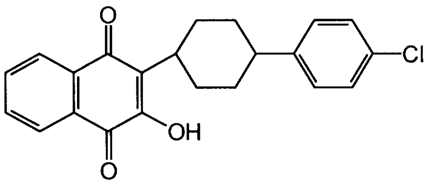
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Buquinolate	4-hydroxy-6,7-diisobutoxy-3-quinolinecarboxylic acid ethyl ester ($C_{20}H_{27}NO_5$) [361.42]	
Decoquinate	6-declyoxy-ethoxy-4-hydroxy-3-quinolinecarboxylic acid ethyl ester ($C_{24}H_{35}NO_5$) [417.53]	
Nequinate	7-(benzyloxy)-6-*n*-butyl-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid methyl ester ($C_{22}H_{23}NO_4$) [365.43]	
Buparvaquone	2-*trans*(4-*t*-butylcyclohexyl)methyl-3-hydroxy-1,4-naphthoquinone ($C_{21}H_{26}O_3$) [326.44]	
Parvaquone	2-cyclohexyl-3-hydroxy-1,4-naphthoquinone ($C_{16}H_{16}O_3$) [256.30]	
Atovaquone	2-[*trans*-4-(4-chlorophenyl)cyclohexyl]-3-hydroxy-1,4-naphthoquinone ($C_{22}H_{19}ClO_3$) [366.69]	

Figure 42.6 Hydroxyquinolones and naphthoquinones.

from the intestinal tract, and what is absorbed is rapidly cleared from the blood and tissues and is only minimally excreted in the milk (Quintero de Leonardo et al., 2008). Evidence indicates that decoquinate can have an anticoccidial effect on first-generation schizonts of *E. tenella*, adversely affect sporulation, and permit the development of immunity if fed at levels that are lower than its coccidiostatic levels (Williams, 1997). This anticoccidial effect also leads to improved feed efficiency and milk production. Activity against *Toxoplasma* and *Cryptosporidium* has also been demonstrated in cattle and sheep.

Decoquinate is licensed and marketed in multiple countries worldwide, including the US, Latin America, Europe, and the Middle East (Taylor and Bartram, 2012). There is some variation between countries in the indications for use and the target species. Formulations in the United States are approved for chickens, calves, beef cattle, and nonlactating dairy cattle, either as a monotherapy, or combined with other drugs including chlortetracycline, lincomycin, tylan, or monensin. Since extralabel use of feed additives is prohibited by the FDA, use in other species in the US is not allowed in this manner.

Clinical Use

Chickens: Decoquinate is used for prevention of coccidiosis caused by *Eimeria* sp. in broilers. It should be fed for at least 28 days when development of coccidiosis is likely. Do not feed to laying chickens. No withdrawal is required.

Cattle: In cattle, decoquinate is used to prevent coccidiosis caused by *Eimeria* sp. when fed for at least 28 days. In beef cattle, no withdrawal time is required. For treatment of cryptosporidiosis, doses of 2 mg/kg were not effective at decreasing oocyst shedding or clinical signs in experimentally challenged calves (Moore et al., 2003). However, treatment of pregnant cows with 1.25 mg/kg/day in the feed for 30 days prior to and for 8 days after parturition prevented clinical signs in the calves.

Sheep: Decoquinate is fed to sheep to provide 0.5 mg/kg BW for at least 28 days to prevent coccidiosis. It is also effective as an aid in the prevention of abortions and perinatal losses due to toxoplasmosis when fed at a dosage of 2 mg/kg BW daily, starting 14 weeks prior to lambing.

Goats: Decoquinate is fed to goats to provide 0.5 mg/kg BW for at least 28 days to prevent coccidiosis. It also reduces clinical cryptosporidiosis in goats fed medicated milk replacer.

Dogs: For preventing clinical relapses of *Hepatozoon americanum* infection, decoquinate feed additive (27.2 grams decoquinate per pound of premix) can be added to moist dog food at a rate of 0.5–1.0 tablespoons per 10 kg BW twice a day (Macintire et al., 2001). Decoquinate has been shown to be effective in resolving clinical signs of infection in a dog diagnosed with severe myositis associated with *Sarcocystis* sp. infection where the drug was used at a dose of 10–20 mg/kg BW orally twice a day (Sykes et al., 2011).

Horses: Decoquinate has been used for the treatment of equine protozoal myeloencephalitis (EPM) caused by *Sarcocystis neurona* at a dose of 0.5 mg/kg orally once daily, combined with levamisole (1 mg/kg) for 10 days total. Improvement in clinical signs with this combination has been noted (Ellison and Lindsay, 2012) and in vitro testing has shown decoquinate to have a cidal effect on developing shizonts of *S. neurona* at concentrations of 240 nM (Lindsay et al., 2013). There are no commercially available, approved combination products and studies have used compounded drug. Further information is necessary before this treatment can be recommended.

Atovaquone

Atovaquone (Mepron® suspension) is a hydroxyquinolone that has broad-spectrum antiprotozoal activity. It is a yellow crystalline solid that is practically insoluble in water. Atovaquone is supplied as a bright yellow suspension of microfine particles at a concentration of 150 mg atovaquone/ml. It is highly lipophilic and administering it with food increases its absorption twofold. It was originally developed for human use to treat drug-resistant strains of malaria and *Pneumocystis carinii* pneumonia. It is discussed here because it was also found to have excellent activity against *T. gondii* and *Babesia* sp. It also has activity against *Eimeria* spp. Atovaquone is thought also to work via inhibition of electron transport at the mitochondrial bc1 complex because cross-resistance often develops between atovaquone and decoquinate (Pfefferkorn et al., 1993).

Clinical Use

Dogs: Atovaquone in combination with azithromycin is the treatment of choice for canine babesiosis (Birkenheuer, 2014). It was administered (13.3 mg/kg PO q 8 h) concurrently with azithromycin (10 mg/kg PO q 24 h) for 10 consecutive days and was shown to completely eliminate *Babesia gibsoni* infection from 8 of 10 dogs treated (Birkenheuer et al., 2004). The dogs in that study had previously been treated unsuccessfully with imidocarb or diminazene. Decreased susceptibility of *B. gibsoni* to treatment with atovaquone in some dogs is associated with a single nucleotide polymorphism in the *B. gibsoni* mitochondrial cytochrome b gene sequence (Matsuu et al., 2006).

Cats: In cats diagnosed with *Cytauxzoon felis*, atovaquone (15 mg/kg PO q 8 h) administered concurrently with azithromycin (10 mg/kg PO q 24 h) for a duration of 10 days was shown to significantly increase survival compared to cats treated with imidocarb dipropionate (3.5 mg/kg given intramuscularly once and then repeated 7 days later) (Cohn et al., 2011).

Parvaquone and Buparvaquone

Parvaquone (Clexon®) and buparvaquone (Butalex®) are naphthoquinones that are used for the treatment of theileriosis. They act on the macroschizonts and intraerythrocytic piroplasms.

Theileriosis is a severe disease of cattle in Africa and is caused by *Theileria parva*, a piroplasm. Several *Theileria* spp. infect wild ruminants in the United States, but none of these causes serious illness.

Clinical Use

Cattle: Parvaquone is administered IM at 20 mg/kg BW as a single treatment for theileriosis. Buparvaquone is given IM at 2.5 mg/kg BW for one or two treatments. Both drugs are considered curative for *Theileria* infections in cattle (Muraguri et al., 1999).

Cats: Parvaquone and buparvaquone have been used to treat *Cytauxzoon felis* infections in cats, although their efficacy is questionable (Kier, 1990).

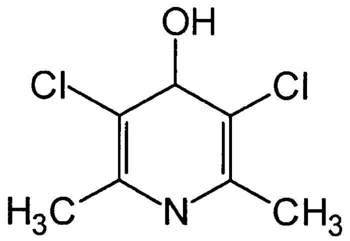
Pyridinols

Spectrum: Coccidiosis, *Leucocytozoon* sp.

Drugs included: Clopidol (Figure 42.7).

Clopidol

Clopidol (Coyden 25®) is the only pyridinol to be used as an anticoccidial. It is practically insoluble in water. It is active against the sporozoite stage, allowing host cell penetration but not parasite development. It also has activity against second-generation schizogony, gametogony, and sporulation. Sporozoites can resume development after the medication is removed. Little anticoccidial immunity develops in chickens receiving this agent. Long (1993) suggested that the mode of action of clopidol was similar to that of the quinolone anticoccidials because of similar structure and biological activity of the agents. However, cross-resistance between clopidol and quinolone anticoccidials does not occur (Long, 1993).



Clopidol

($C_7H_7Cl_2NO$)

[192.06]

Figure 42.7

Clinical Use

Chickens and turkeys: Clopidol is fed at 0.0125–0.025% for prevention of coccidiosis. A 5-day withdrawal is required if the 0.025% level is used. This level may be lowered to 0.0125% 5 days before withdrawal. Clopidol is transmitted to the eggs of hens that are fed clopidol-containing diets (Long, 1971). Clopidol is also approved for the prevention of *Leucocytozoon smithi* infections in turkeys when fed at its anticoccidial levels.

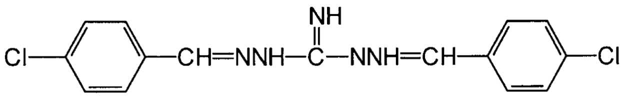
Guanidine Derivatives

Spectrum: Coccidiosis.

Drugs included: Robenidine (Figure 42.8).

Robenidine

Robenidine (Cycostat®, Robenz®) is a synthetic anticoccidial derivative of guanidine. It is active against the first-generation schizont of *E. tenella* by preventing formation of merozoites. Feeds with robenidine should not be given to laying hens, as studies have shown that robenidine is transmitted to the eggs (Long et al., 1981), and these eggs may have an unpleasant taste even though the ability of humans to taste robenidine is apparently under genetic



Robenidine

($C_{15}H_{13}Cl_2N_5$)

[334.21]

Figure 42.8

control. It does not have any other adverse effects on egg production or quality.

Clinical Use

Chickens: Robenidine is used for the prevention of coccidiosis in chickens. Finish feeds must be fed within 50 days of manufacture. Addition of bentonite clays to feeds may decrease the activity of robenidine. A 5-day withdrawal is required. If robenidine is not withdrawn 5 days before slaughter, the flesh of medicated birds will have an unpleasant taste.

Rabbits: Robenidine can be used for the prevention of coccidiosis caused by *Eimeria* sp. in rabbits when fed at 0.75–1.00 kg per ton of complete feed to supply 50–66 ppm robenidine hydrochloride. Use at these feed levels carries a 5-day withdrawal time.

Thiamine Analogues

Spectrum: Coccidiosis.

Drugs included: Amprolium (Figure 42.9).

Amprolium

Amprolium (Corid®, Amprol®) is approved for use in the United States as a preventative and treatment of *Eimeria* infections in chickens, turkeys, pheasants, and calves. It is one of the most commonly administered anticoccidial agents in veterinary medicine. It is structurally related to vitamin B_1 (thiamine) and the antiparasitic activity of the drug is thought to be related to competitive inhibition of active thiamine transport into the parasite. There is a 50-fold greater sensitivity of the parasite system compared with the host system. However, long-term or high-dose administration of amprolium can lead to clinical thiamine deficiency in treated animals, resulting in cerebrocortical necrosis (polioencephalomalacia).

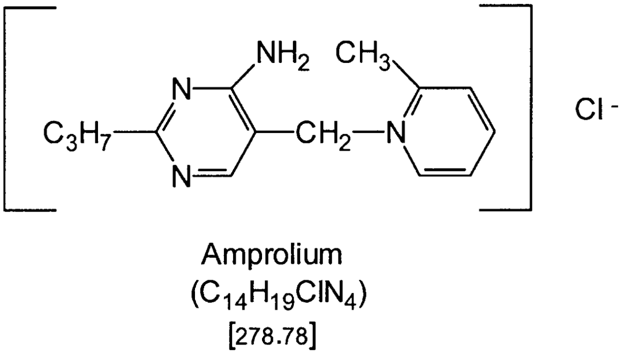


Figure 42.9

Amprolium is freely soluble in water. Because amprolium lacks the hydroxyethyl function of thiamine it is not phosphorylated to a pyrophosphate analog (Looker et al., 1986). Amprolium acts on the first-generation schizont to prevent merozoite production and has some activity against sexual stages and the sporulating oocyst. Amprolium-resistant strains of chicken and turkey *Eimeria* spp. are prevalent, therefore it is often combined with other agents to increase activity.

Clinical Use

Chickens and turkeys: Amprolium is used for the prevention of coccidiosis in chickens as a feed additive or in the drinking water. No withdrawal is required. Amprolium is used for the treatment and prevention of coccidiosis in turkeys when fed continuously.

Pheasants: Amprolium is approved for use in the prevention of coccidiosis in pheasants and is fed continuously. It must not be used in feeds containing bentonite.

Cattle: Amprolium is given to calves in the feed, drinking water, or orally at 5 mg/kg BW for 21 days to prevent coccidiosis or at 10 mg/kg BW for 5 days as an aid to treat coccidiosis.

Goats: Amprolium is used for the treatment of pathogenic *Eimeria* sp. in goats. Resistance is common and increasing, therefore higher doses are recommended. Doses of 50 mg/kg orally once daily for 5 days effectively reduced shedding of oocysts whereas 10 mg/kg did not (Young et al., 2011).

Dogs: In dogs, amprolium can be used for the treatment of coccidiosis at 100–200 mg total dose (20% powder) orally once a day for 7–12 days. Amprolium is also used in the drinking water (sole source) at 30 ml (9.6% solution)/gal for up to 10 days or in the food at 250–300 mg (20% powder) once a day for 7–12 days.

Cats: Amprolium can be used for the treatment of coccidiosis at 110–220 mg/kg orally once a day for 7–12 days. Amprolium is also used in the drinking water (sole source) at 1.5 teaspoons (9.6% solution)/gal for up to 10 days. Amprolium can be combined with sulfadimethoxine and used for the treatment of coccidiosis at 150 mg/kg BW amprolium and 25 mg/kg BW sulfadimethoxine once a day for 14 days.

Nitrobenzamides

Spectrum: Coccidiosis.

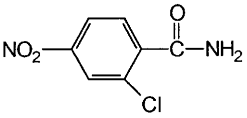
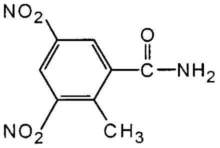
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Aklomide	2-chloro-4-nitrobenzamide ($C_7H_5ClN_2O_3$) [200.60]	
Dinitolmide	2-methyl-3,5-dinitrobenzamide ($C_8H_7N_3O_5$) [225.16]	

Figure 42.10 Nitrobenzamides.

Drugs included: Aklomide, dinitolmide (Figure 42.10).

Aklomide and Dinitolmide

Aklomide and dinitolmide (Zoamix®) are nitrobenzamide anticoccidial agents. These agents act primarily on the first-generation schizonts, and dinitolmide inhibits sporulation of oocysts (Mathis and McDougald, 1981). Dinitolmide is coccidiostatic if given for 6 days but is coccidiocidal if given for longer periods (Long, 1993). Nitrobenzamide-resistant strains of coccidia are common. The anticoccidial mode of action of nitrobenzamides is not known.

Aklomide is not currently marketed as a single agent or combined with other anticoccidial drugs in the United States.

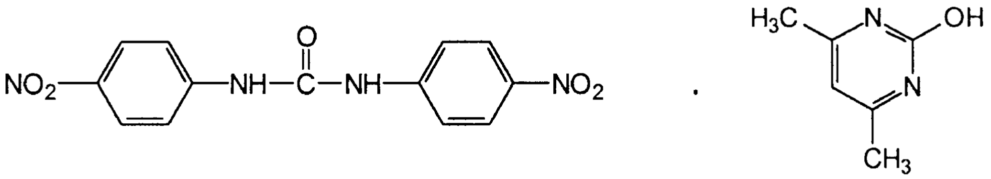
Clinical Use

Chickens and turkeys: Dinitolmide is fed as a single agent to prevent coccidiosis in chickens. Do not feed to layers or birds over 14 weeks of age. Dinitolmide is also approved for use in nonlaying turkeys in feed in birds up to 14–16 weeks of age. Combination products are also available.

Nicarbazin

Spectrum: Coccidiosis.

Nicarbazin (Nicarb®, Cycarb®) is an equimolecular complex of 4,4′-dinitrocarbanilide and 2-hydroxy-4,6-dimethylpyrimidine (Figure 42.11). The agents are absorbed separately from the digestive tract, and both are needed for anticoccidial activity. Dry crystals are strongly electrostatic and present some dry-mixing problems. The precise mode of action of nicarbazin is not known. Nicarbazin causes reduced egg production, depressed egg weight, and reduced eggshell thickness and egg-yolk mottling when fed to white leghorn layers at 125 ppm (Jones et al., 1990). It also causes poor hatchability and depigmentation of brown-shelled eggs. Nicarbazin is usually restricted in use to the starting period because of potential growth-suppressing effects and to cooler months of the year because of its potential to enhance the effects of heat distress (McDougald, 1993).



Nicarbazin
($C_{19}H_{18}N_6O_6$)
[426.38]

Figure 42.11

Clinical Use

Chickens: Nicarbazin is fed for the prevention, but not the treatment, of coccidiosis. It is not labeled for laying hens. A combination of narasin and nicarbazin (Maxiban®) is available for prevention of coccidiosis in broilers. It is for broilers only and should not be fed to other types of chickens. The use of this combination has been associated with increased mortality of broilers in times of heat distress. Feed containing this combination may cause fatalities in horses that ingest it, and it should not be fed to adult turkeys.

Alkaloids

Spectrum: Coccidiosis, theileriosis.

Drugs included: Halofuginone (Figure 42.12).

Halofuginone

Halofuginone (Stenerol®) is an alkaloid originally isolated from the plant *Dichroa febrifuga*. Halofuginone is active against the asexual stages of coccidia. Its anticoccidial mode of action is not known. Halofuginone is transmitted to eggs from hens that have been fed halofuginone for 1 week (Long et al., 1981), but no adverse effects on egg production or egg quality have been associated with administration (Jones et al., 1990). Halofuginone has been associated with skin tears in chickens, and it has been shown to be an inhibitor of collagen type I synthesis in avian and mammalian cells (Granot et al., 1993). Halofuginone is toxic to fish and other aquatic life and must not be fed to waterfowl. Halofuginone is a skin and eye irritant and appropriate precautions (such as protective clothing) should be taken when handling this agent.

Clinical Use

Chickens and turkeys: Halofuginone is used for the prevention of coccidiosis in chickens when administered in feed. Do not feed to layers. Halofuginone is also approved for the prevention of coccidiosis in growing turkeys.

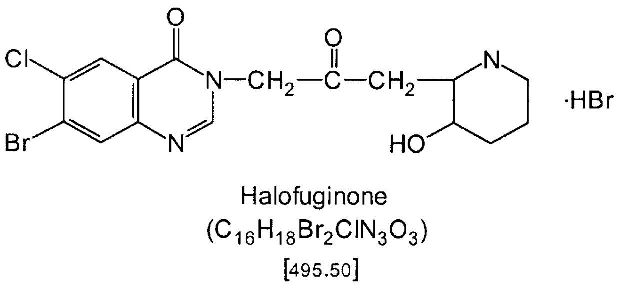


Figure 42.12

Cattle: In cattle, halofuginone is given orally at 1–2 mg/kg BW for a single treatment and is curative for *Theileria* infections. At 2 mg/kg BW transient diarrhea may occur. Halofuginone is given orally at 100 μg/kg BW orally once a day for 7 days as a treatment or preventative for *Cryptosporidium* spp. infections in calves. While some studies indicate that lower numbers of oocysts and decreased severity of diarrhea can be observed in treated calves, others have identified no clinical benefit (Trotz-Williams et al., 2011; Klein, 2008). The utility of halofuginone is likely influenced by multiple factors. Halofuginone is more effective at reducing clinical disease and decreasing shedding of *Cryptosporidium* in calves when calves are housed individually (De Waele et al., 2010) and when no other concurrent infections are present (Meganck et al., 2015).

Polyether Ionophores

Spectrum: Coccidiosis.

Drugs included: Lasalocid, maduramicin, monensin, narasin, semduramicin, salinomycin (Figure 42.13).

The polyether ionophore antibiotics were first discovered in the early 1950s, and their anticoccidial activities were recognized in the late 1960s. Because of their broad spectrum of activity and the development of drug resistance to other agents, the ionophores gained widespread usage in the poultry industry soon after their introduction. Polyether ionophores fall into one of five classes: monovalent, monovalent glycosides, divalent, divalent glycosides, or divalent pyrrole ethers. The mode of action of ionophores is related to their ability to form lipophilic complexes with alkali metal cations and to transport these cations across biological membranes. Different ionophores may produce varying affinities for different cations. Ionophores that have anticoccidial activity are thought to act against extracellular sporozoites and merozoites. Extracellular stages develop membrane blebs indicating alterations in membrane integrity and in internal osmolality. Because coccidia have no osmoregulatory organelles, this change in internal osmotic conditions damages the parasites.

Because of the unique mode of action of ionophores, development of anticoccidial resistance to ionophores was difficult to produce under experimental conditions and slow to develop after clinical use in the field. In the mid to late 1980s, ionophore-resistant strains of chicken *Eimeria* spp. were documented in the United States, and ionophore resistance is now common. Cross-resistance between ionophores is common, although strain differences in coccidial response to specific ionophores have been demonstrated. In general, resistance to a monovalent polyether ionophore confers some cross-resistance

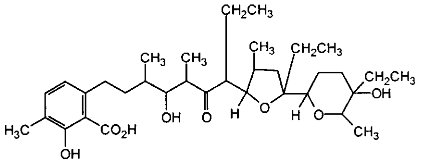
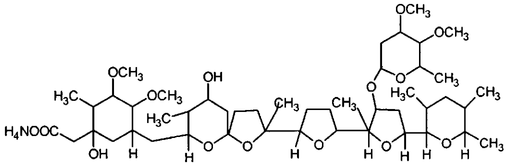
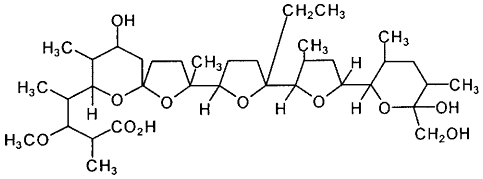
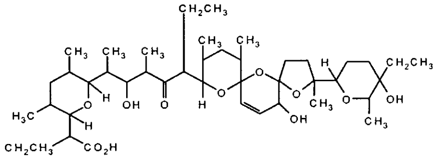
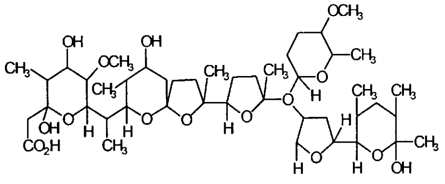
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Lasalocid	6-[7*R*-[5*S*-Ethyl-5-(5*R*-ethyltetra-hydro-5-hydroxy-6 *S*-methyl-2*H*-pyran-2*R*-yl) tetrahydro-3*S*-methyl-2*S*-furanyl]-4*S*-hydroxy-3*R*,5*S*-dimethyl-6-oxonon-yl]-2-hydroxy-3-methylbenzoic acid $(C_{34}H_{54}O_8)$ [590.80]	
Maduramicin	(3*R*,4*S*,5*S*,6*R*,7*S*,22*S*)-23,27-Didemethoxy-2,6,22-tridemethyl-11-*O*-demethyl-22-[(2,6-dideoxy-3,4-di-*O*-methyl-β-Larabinohexopyranosyl)oxy]-6-methyloxylonomycin A monoammonium salt $(C_{47}H_{83}NO_{17})$ [934.17]	
Monensin	2-[5-Ethyltetrahydro-5-[tetrahydro-3-methyl-5-[tetrahydro-6-hydroxy-6-(hydromethyl)-3,5-dimethyl-2*H* -pyran-2-yl]-2-furyl]-2-furyl]-9-hydroxy-β-methoxy-α, γ, 2,8-tetramethyl-1,6-dioxaspiro [4.5] decane-7-butyric acid $(C_{36}H_{62}O_{11})$ [670.90]	
Narasin	(αβ,2β,3α,5α,6α)-α-ethyl-6-[5-[5-(5α-ethyltetrahydro-5β-hydroxy-6α-methyl-2*H*-pyran-2β-yl)-3″α,4,4″,5,5″α,6″-hexahydro-3′β-hydroxy-3″β,5α,5″β-trimethylspiro] furan-2(3*H*),2′-[2*H*]pyran-6′(3′*H*),2″-[2*H*]pyran]6″α-yl]2α-hydroxy-1α,3β-dimethyl-4-oxoheptyl]-tetrahydro-3,5-dimethyl-2*H*-pyran-2-acetic acid $(C_{43}H_{72}O_{11})$ [765.05]	
Semduramicin	(2*R*,3*S*,4*S*,5*R*,6*S*)-tetrahydro-2,4-dihydroxy-6-[(1*R*)-1-[(2*S*,5*R*,7*S*,8*R*,9*S*)-9-hydroxy-2,8-dimethyl-2-[(2*R*,6*S*)-tetrahydro-5-methyl-5-[(2*R*,3*S*,5*R*)-tetrahydro-5[(2*S*,3*S*,5*R*,6*S*)-tetrahydro-6-hydroxy-3,5,6-trimethyl-2*H*-pyran-2-yl]-3-[[(2*S*,5*S*,6*R*)-tetrahydro-5-methoxy-6-methyl-2*H*-pyran-2- yl]oxy]-2-furyl]-2-furyl]-1,6-dioxaspirol [4.5]dec-7-yl]ethyl]-5-methoxy-3-methyl-2*H*-pyran-2-acetic acid $(C_{44}H_{77}O_{16})$ [748.47]	

Figure 42.13 Ionophores antibiotics.

to other monovalent polyether ionophores, but susceptibility to monovalent monoglycoside and divalent polyether ionophores may be retained.

Ionophores are potentially toxic for highly susceptible species. Horses appear to be the most sensitive species, with a monensin LD_{50} of 1.4 mg/kg, compared with goats (LD_{50} 26.4 mg/kg) and chickens (LD_{50} 214 mg/kg) (Deljou et al., 2014). Clinical signs occur from acute myocardial and muscle degeneration. Sweating, colic, and ataxia with hindlimb paresis/paralysis are also noted. Pathologically, focal degenerative cardiomyopathy, skeletal muscle necrosis, and congestive heart failure may be noted. Ionophore toxicosis can be potentiated with concurrent administration of tiamulin, chloramphenicol, macrolides, sulfonamides, and cardiac glycosides (Novilla, 1992; Mitema et al., 1988). Exposure of horses to ionophores is usually accidental, or resulting from horses gaining access to medicated feed, particularly through feed mill errors. Care should always be taken to prevent highly susceptible animals from gaining access to feeds containing these products.

Lasalocid

Lasalocid (Avatec®, Bovatec®) is a divalent polyether ionophore that is a fermentation product of *Streptomyces* *lasaliensis* and was the second ionophore marketed in the United States. Lasalocid is well tolerated when fed with tiamulin (Meingasser et al., 1979). Lasalocid is transmitted to eggs of hens that have been fed this medication for 1 week.

Clinical Use

Chickens and turkeys: Lasalocid is fed for the prevention of coccidiosis in chickens. Lasalocid is also approved for the prevention of coccidiosis in growing turkeys.

Chukar partridges: Lasalocid is approved for the prevention of coccidiosis in chukar partridges and is fed until the birds are 8 weeks old.

Cattle: In cattle, lasalocid is used to prevent coccidiosis and is fed at 1 mg/kg BW, with a maximum of 360 mg/animal/day. Do not use in breeding animals.

Sheep: Lasalocid is fed in the ration to sheep to provide 15–70 mg/animal/day and is used in the prevention of coccidiosis. Do not administer to breeding animals.

Rabbits: Lasalocid is approved for use in the prevention of hepatic coccidiosis in rabbits and is fed until the rabbits are 6.5 weeks old.

Maduramicin

Maduramicin (Cygro®) is a monovalent monoglycoside polyether ionophore that is a fermentation product of *Actinomadura yumaense*. Maduramicin does not adversely affect egg production or egg quality (Jones et al., 1990). Maduramicin is well tolerated when fed with tiamulin (Meingasser et al., 1979). At higher doses (≥6 ppm), an adverse effect on growth and feathering may occur.

Clinical Use

Chickens: Maduramicin is used for the prevention of coccidiosis in broilers and fed at 5 ppm. It is for broilers only and should not be fed to other types of chickens.

Monensin

Monensin (Coban®, Rumensin®) is a monovalent polyether ionophore that is a fermentation product of *Streptomyces cinnamonensis*. Tiamulin may interfere with the metabolism of monensin in chickens and cause weight suppression (Meingasser et al., 1979). Monensin is apparently not transmitted to eggs of hens fed monensin for 1 week, nor does it adversely affect egg production or quality. Mature turkeys and guinea fowl should not have access to monensin-containing diets. Monensin will cause deaths in horses and guinea fowl that ingest feeds containing it.

Clinical Use

Chickens and turkeys: Monensin is fed for prevention of coccidiosis in broilers. Do not feed to layers or chickens over 16 weeks of age. Monensin is used for prevention of coccidiosis in turkeys. Do not feed to turkeys over 10 weeks old.

Bobwhite quail: Monensin is approved for use in bobwhite quail for prevention of coccidiosis and is fed at 73 g/ton from 1 day to 10 weeks old.

Cattle: In cattle, monensin is used to prevent coccidiosis and is fed to provide 100–360 mg/animal/day. It has recently been approved for use in dairy cattle as well, with a zero meat and milk withdrawal time.

Goats: Monensin is fed at 20 g/ton for the prevention of coccidiosis in confinement-reared goats. Do not feed to goats producing milk for human consumption.

Narasin

Narasin (Monteban®) is a monovalent polyether ionophore that is a fermentation product of *Streptomyces aureofaciens*. It is structurally similar to salinomycin, differing only in the presence of a methyl group in narasin that is not present in salinomycin. It does not adversely affect egg production or egg quality. Narasin may cause fatalities in horses that ingest it, and it should not be fed to adult turkeys. Tiamulin may interfere with the metabolism of narasin in chickens and cause weight suppression (Meingasser et al., 1979).

Clinical Use

Chickens: Narasin is a feed additive for the prevention of coccidiosis in broilers. It is for broilers only and should not be fed to other types of chickens.

Semduramicin

Semduramicin (Aviax®) is a monovalent monoglycoside polyether ionophore that is a fermentation product of a mutant of *Actinomadura roseorufa* (Ricketts et al., 1992). The parent produced a diglycoside form of semduramicin that had to be semisynthetically modified. Both forms of the monoglycoside agent have identical activity. It is well tolerated when coadministered with tiamulin (Ricketts et al., 1992).

Clinical Use

Chickens: Semduramicin is used as a feed additive for prevention of coccidiosis in broiler chickens.

Salinomycin

Salinomycin (Bio-Cox®) is a monovalent polyether ionophore that is a fermentation product of *Streptomyces albus* and was the third ionophore marketed in the United States. Salinomycin is active against sporozoites and early and late asexual stages of chicken coccidia (Conway et al., 1993). Salinomycin does not adversely affect egg production or egg quality. Tiamulin may interfere with the metabolism of salinomycin in chickens and cause weight suppression (Meingasser et al., 1979). It is not for use with pellet binders such as bentonite clays. Salinomycin will cause deaths in horses and adult turkeys that ingest feeds containing it. Toxicity associated with salinomycin in horses and cats has been related to predominately neurological signs, although elevations in muscle enzymes are often present (Aleman et al., 2007; Pakozdy et al., 2010).

Clinical Use

Chickens: Salinomycin is fed for prevention of coccidiosis in broilers. Do not feed to layers.

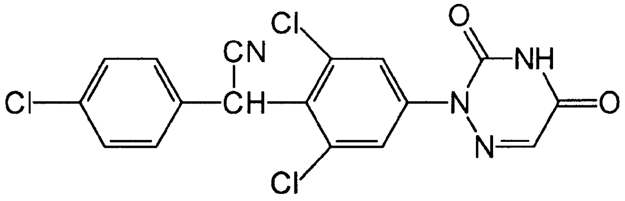
Bobwhite quail: Salinomycin is approved for prevention of coccidiosis in bobwhite quail when administered in feed.

Triazine Derivatives

Spectrum: *Sarcocystis neurona*, coccidiosis, toxoplasmosis, *Neospora caninum*, *Neospora hughesii*.

Drugs included: Diclazuril, toltrazuril, ponazuril (Figures 42.14, 42.15). The triazine antiprotozoal drugs act on the apicoplast, a plastid obtained by endosymbiosis that is present in apicomplexan parasites, but is not present in the vertebrate host. The exact function of the apicoplast is not known; however, it may play a vital role in biosynthesis of amino acids and fatty acids, assimilation of nitrate and sulfate, and starch storage, as has been demonstrated for other plastid organelles (Kühler et al., 1997; Fichera and Roos, 1997).

Diclazuril



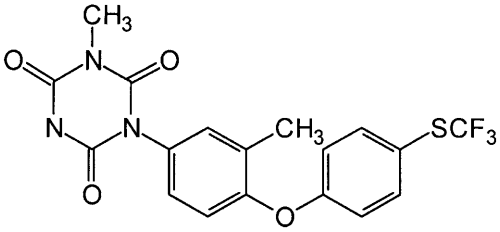
Diclazuril
($C_{17}H_9Cl_3N_4O_2$)
[407.64]

Figure 42.14

Diclazuril (Clinicox®, Protazil®) is a benzeneacetonitrile that has potent anticoccidial activity when fed at low levels in the feed. The mechanism of action against *Sarcocystis neurona* is to inhibit merozoite production.

Clinical Use

Horses: Diclazuril has been used as a treatment for equine protozoal myeloencephalitis (EPM). Doses of 5 mg/kg (equivalent to 500 grams Clinicox®/ 450-kg horse) have been successful in improving clinical signs of EPM using a shorter duration of treatment (30 days) compared to therapy with combinations of sulfonamides and pyrimethamine (90–120 days). However, because of the large volume of drug to be administered, some horses have to be given the drug via a nasogastric tube, which makes treatment inconvenient and difficult. Approval of an alfalfa-based pelleted formulation (Protazil™, 1.56%) has made treatment with diclazuril in horses much more practical because it can be added as a top-dressing to feed for repeated dosing. The pharmacokinetics have been well defined in horses (Dirikolu et al., 1999; Hunyadi et al., 2015; Hunyadi L, Papich MG, Pusterla N, unpublished data). In the study by Dirikolu et al. (1999) after a dose of 2.5 grams per 450 kg (as Clinacox®) it had a half-life of approximately 43 hours and maintained concentrations at 7–9 µg/ml with daily doses for 21 days. Cerebrospinal fluid (CSF) concentrations were



Toltrazuril
($C_{18}H_{14}F_3N_3O_4S$)
[425.38]

Figure 42.15

approximately 15% of plasma concentrations. Concentrations believed to be required to inhibit *Sarcocystis neurona* are 1 ng/ml. In more recent studies (Hunyadi et al., 2015; Hunyadi L, Papich MG, Pusterla N, unpublished data) the pelleted medication designed for top-dressing horse feed (Protazil$^{\mathrm{TM}}$, 1.56%) was administered to horses at the approved dose of 1 mg/kg and at a lower dose of 0.5 mg/kg. The half-life was approximately 87 and 55 hours from the low and high dose, respectively. After multiple doses to steady-state once daily, the half-life was 72 hours and 54 hours from the low and high dose, respectively, and the plasma drug concentrations were not different between the low dose and high dose. CSF concentrations were 5.7% of the plasma concentrations at the low dose. Because of the long half-life, and no difference between 0.5 mg/kg and 1.0 mg/kg, a follow-up study was performed in which it was shown that a dosing schedule of every 3 or 4 days of 0.5 mg/kg of the pelleted formulation would maintain concentrations above the desired threshold in horses to inhibit *Sarcocystis* (Hunyadi L, Papich MG, Pusterla N, unpublished data).

Chickens: Diclazuril is approved for use in broilers as a preventative for coccidiosis. There are several other combination products available as well. The withdrawal times can vary, depending on the product. This drug is not approved for use in laying hens.

Turkeys: Diclazuril is approved for use in the prevention of coccidiosis in turkeys. It is also available combined with bacitracin. These products should not be fed to breeding turkeys or layers.

Rabbits: When fed at 1–2 ppm continuously in pelleted feed, diclazuril was effective in controlling the clinical signs of intestinal coccidiosis in experimentally infected rabbits (Vanparijs et al., 1989).

Toltrazuril

Toltrazuril (Baycox®, Procox®) is a triazinon drug that has broad-spectrum anticoccidial and antiprotozoal activity. It is not commercially available in the United States, but it is available in other countries. It is active against both asexual and sexual stages of coccidia by inhibiting nuclear division of schizonts and microgamonts and the wall-forming bodies of macrogamonts. It may also be useful in the treatment of neonatal porcine coccidiosis, EPM, and canine hepatozoonosis.

Clinical Use

Swine: Toltrazuril has been shown to reduce the signs of coccidiosis in naturally infected nursing pigs when a single oral 20–30 mg/kg BW dose is given to 3 to 6-day-old pigs (Driesen et al., 1995). Clinical signs were reduced from 71 to 22% of nursing pigs, and diarrhea and oocyst excretion were also decreased by the single oral treatment. Approved products carry a 77-day withdrawal time in the United Kingdom.

Calves and lambs: Toltrazuril is used for the prevention of clinical signs of coccidiosis and reduction of coccidia shedding in calves and lambs as a single dose treatment. Withdrawal times in the United Kingdom are 63 and 42 days for calves and lambs, respectively.

Dogs: For hepatozoonosis, toltrazuril given orally at 5 mg/kg BW every 12 hours for 5 days or given orally at 10 mg/kg BW every 12 hours for 10 days caused remission of clinical signs in naturally infected dogs in 2–3 days (Macintire et al., 2001). Unfortunately, most treated dogs relapsed and eventually died from hepatozoonosis. In puppies with *Isospora* sp. infection, treatment with 0.45 mg emodepside in combination with 9 mg/kg BW toltrazuril (Procox®, Bayer Animal Health) reduces fecal oocyst count by 91.5–100%. There was no difference in duration of diarrhea when treatment was begun after the onset of clinical signs during patent infection (Altreuther et al., 2011).

Cats: In kittens experimentally infected with *Isospora* spp., treatment with a single oral dose of 0.9 mg emodepside in combination with 18 mg/kg BW toltrazuril (Procox®, Bayer Animal Health) reduces oocyst shedding by 96.7–100% if given during the prepatent period (Petry et al., 2011).

Horses: Toltrazuril has also been used for the treatment of EPM. This drug is safe, even at high doses. Current recommended treatments are 5–10 mg/kg orally for 28 days. Despite the favorable efficacy with toltrazuril, its use has diminished in horses because of better availability of other effective drugs.

Ponazuril

Ponazuril (Marquis®), also known as toltrazuril sulfone, is an active metabolite of toltrazuril; therefore, its mechanism of action is identical to that of toltrazuril. It is available in a paste formulation for horses. It has activity against *Sarcocystis neurona, Toxoplasma gondii, Isospora suis*, and *Neospora caninum.*

Clinical Use

Horses: Ponazuril is approved for use in horses as a treatment for EPM at a dose of 5 mg/kg orally for 28 days. Treatment can be extended for another 28 days if no improvement is seen. A loading dose of 15 mg/kg orally once is recommended to attain therapeutic plasma and CSF concentrations for *S. neurona* after a single dose. Successful treatment of EPM caused by *N. hughesii* with ponazuril has also been described (Finno et al., 2007). No adverse effects have been noted. Intermittent administration of ponazuril at 20 mg/kg orally once a week has been recommended as a preventative dosing for EPM, as it significantly decreased intrathecal anti-*S. neurona* antibody responses in horses inoculated with *S. neurona* sporocysts (MacKay et al., 2008).

Cattle: Ponazuril has been shown to be effective at a dose of 20 mg/kg orally once a day for 6 days in calves experimentally infected with *Neospora caninum*.

Swine: There are no approved ponazuril products for use in swine; however, the equine formulation has good oral bioavailability in swine and is used extralabel for treatment of coccidiosis caused by *Isospora suis*. Unlike the other triazine drugs, ponazuril is cidal against all intracellular and developmental stages of *Isospora* and it does not interfere with natural immunity (Mehlhorn et al., 1984). A single oral dose of 5 mg/kg to 2 to 3-month-old piglets resulted in a half-life of 5.6 days and detectable plasma concentrations for at least 33 days (Zou et al., 2014). This, coupled with the lack of tissue residue data, mean that any animal treated with ponazuril would require a greatly prolonged withdrawal time.

Dogs and cats: Ponazuril at a dose of 50 mg/kg BW orally once a day for 3 days decreases excretion of *Cystoisospora* spp. oocysts below the detection limit in 92.9% and 87.5% of treated shelter dogs and cats, respectively. Animals with high fecal oocyst counts were significantly more likely to remain infected and to require a second treatment course (Litster et al., 2014).

Sulfonamides

Spectrum: Coccidiosis.

Drugs included: Sulfaguanidine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamethazine (syn. sulfadimidine), sulfamethoxazole, sulfanitran, and sulfaquinoxaline (Figure 42.16).

Sulfonamides were the first effective anticoccidials used. Sulfonamides can be either coccidiostatic or coccidiocidal. The pharmacology of sulfonamides is also discussed in Chapter 32. The structure of sulfonamides is similar to para-aminobenzoate (para-aminobenzoic acid, PABA), which is required by bacteria in the synthesis of folate (folic acid). Sulfonamides interfere in the early phases of folate synthesis. Mammalian and avian cells use preformed folate and are therefore not affected by the sulfonamide treatments. Sulfonamides are often used in combination with dihydrofolate reductase/thymidylate synthase (DHFR/TS) inhibitors such as trimethoprim, pyrimethamine, or ormetoprim, because of the observed synergistic effects due to activity at two places in folate biosynthesis. Sulfonamides have most activity against the asexual stages and lesser activity against the sexual stages of coccidia.

Sulfonamides used in veterinary medicine for the treatment or prevention of coccidia and coccidia-like parasites include sulfaguanidine, sulfadiazine, sulfadimethoxine, sulfadoxine, sulfamethazine (in some countries called *sulfadimidine*), sulfamethoxazole, sulfanitran, and sulfaquinoxaline. Many are used in combination with other products that have antibacterial or growth-promoting properties. Combinations with trimethoprim or ormetoprim are discussed in Section Dihydrofolate Reductase/Thymidylate Synthase Inhibitors.

Clinical Use

Chickens and turkeys: Sulfamethazine is approved for use in chickens and turkeys for the treatment of coccidiosis when administered in drinking water. Sulfamethazine should not be administered to animals producing eggs for human consumption.

Cattle: Sulfamethazine is used to treat coccidiosis in calves by oral administration of a single dose of 100 mg of sulfamethazine per pound of body weight the first day and 50 mg per pound of body weight on each following day, not to exceed 5 days. Other formulations are also available. Do not feed to preruminating calves or lactating dairy cattle. Sulfaquinoxaline can be administered in the drinking water for 3 to 5 days. Treated animals must actually consume enough medicated water to provide 6 mg per pound body weight per day. Do not feed to calves to be slaughtered under 1 month old or use in lactating dairy cattle. Extralabel use of sulfonamides in adult dairy cattle is prohibited by the FDA.

Dogs and cats: Several agents have been used for the treatment of intestinal coccidiosis in dogs and cats (Lindsay et al., 1997a), but only sulfadimethoxine is approved by the FDA for this usage. Sulfadimethoxine is used for treatment of coccidiosis in dogs and cats at 55 mg/kg BW for 1 day, and then 27.5 mg/kg BW once a day for 14–20 days.

Rabbits: Sulfaquinoxaline is used in the feed of rabbits for 30 days for treatment of coccidiosis.

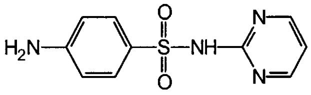
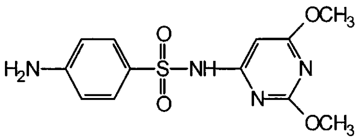
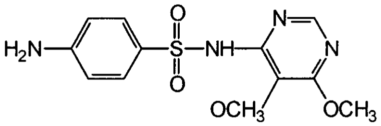
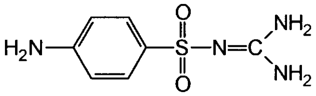
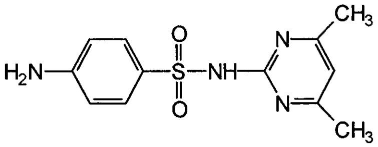
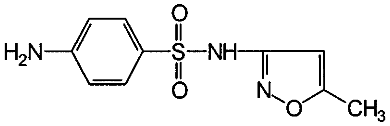
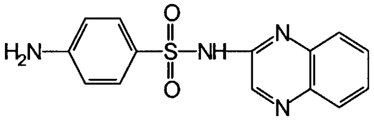
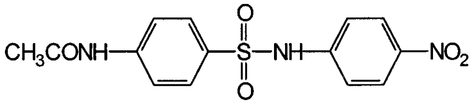
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Sulfadiazine	4-amino-*N*-2-pyrimidinylbenzenesulfonamide ($C_{10}H_{10}N_4O_2S$) [250.28]	
Sulfadimethoxine	4-amino-*N*-(2,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_4S$) [310.33]	
Sulfadoxine	4-amino-*N*-(5,6-dimethoxy-4-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_4S$) [310.34]	
Sulfaguanidine	4-amino-*N*-(aminoiminomethyl)-benzenesulfonamide ($C_7H_{10}N_4O_2S$) [214.24]	
Sulfamethazine	4-amino-*N*-(4,6-dimethyl-2-pyrimidinyl)-benzenesulfonamide ($C_{12}H_{14}N_4O_2S$) [278.32]	
Sulfamethoxazole	4-amino-*N*-(5-methyl-3-isoxazolyl)-benzenesulfonamide ($C_{10}H_{11}N_3O_3S$) [253.31]	
Sulfaquinoxaline	4-amino-*N*-2-quinoxalinyl-benzenesulfonamide ($C_{14}H_{12}N_4O_2S$) [300.33]	
Sulfanitran	4′-[(*p*-nitrophenyl)sulfamoyl]acetanilide ($C_{14}H_{13}N_3O_5S$) [335.34]	

Figure 42.16 Sulfonamides.

Dihydrofolate Reductase/Thymidylate Synthase Inhibitors

Spectrum: Coccidiosis, *Toxoplasma* sp., *Neospora* sp., *S. neurona*, leucocytozoon, *Hepatozoon americanum*.

Drugs included: trimethoprim, ormetoprim, pyrimethamine (Daraprim) (Figure 42.17).

In protozoa, unlike other eukaryotic cells, the dihydrofolate reductase and thymidylate synthase enzymes do not exist as separate molecular entities, but instead these enzymes are part of a bifunctional DHFR/TS complex that has both DHFR and TS activity (Roos, 1993). The best known and most often used members of this group are trimethoprim, ormetoprim, and pyrimethamine. These drugs were previously introduced in Chapter 32.

Trimethoprim

Trimethoprim is available in several formulations, as well as in trimethoprim combinations for oral or parenteral administration. Examples include trimethoprim + sulfadiazine (Tribrissen®, Di-Trim®) and trimethoprim + sulfamethoxazole (Bactrim®, Septra®, human drugs). Trimethoprim also is available as a single drug

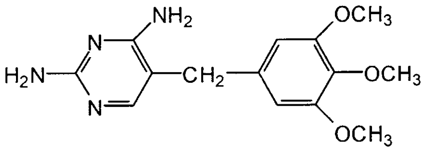
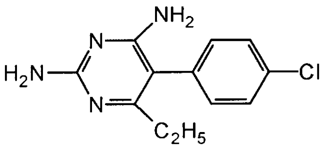
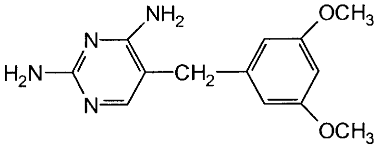
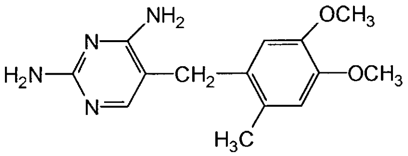
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Trimethoprim	2,4-diamino-5-(3,4,5-trimethoxybenzyl)pyrimidine ($C_{14}H_{18}N_4O_3$) [290.32]	
Pyrimethamine	2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine ($C_{12}H_{13}ClN_4$) [248.71]	
Diaveridine	2,4-diamino-5-veratrylpyrimidine ($C_{13}H_{16}N_4O_2$) [260.29]	
Ormetoprim	2,4-diamino-5-(4,5-dimethoxy-2-methylbenzyl)-pyrimidine ($C_{13}H_{15}N_4O_2$) [259.17]	

Figure 42.17 Dihydrofolate reductase/thymidylate synthase inhibitors.

(Proloprim). Pharmacology of trimethoprim is discussed in more detail in Chapter 32.

Trimethoprim is readily absorbed from the digestive tract after oral administration, and peak serum levels occur 1–4 hours later. The half-life is 3.8 hours in the horse, 3.0 hours in the dog, and 10.6 hours in human beings. Concurrent administration of a sulfonamide does not affect the rate of absorption of trimethoprim. Trimethoprim is widely distributed in tissues. Concentrations of trimethoprim in cerebral spinal fluid are about 40% of those in the serum (Zinner and Mayer, 1990). Trimethoprim is almost completely (60–80%) excreted by the kidneys within 24 hours, and some is also excreted in the bile. The remainder is excreted as metabolites by the kidneys. Use of trimethoprim and other DHFR/TS inhibitors is associated with adverse effects on bone marrow. Administration of folinic acid usually will counteract these adverse effects.

Trimethoprim is available in several tablet, liquid, and paste formulations in combination with sulfamethoxazole (Cotrimethoxazole®, Bactrim®, Septra®) or sulfadiazine (Di-Trim®) that are usually in the ratio of 1 part trimethoprim to 5 parts sulfonamide. In addition to their antibacterial use (Chapter 32), these agents are used in treatment of coccidiosis, toxoplasmosis, neosporosis, EPM, and malaria. Adverse reactions to these agents are similar to those of the individual constituents. Adverse effects from the sulfonamide are more common, but adverse effects from trimethoprim are also possible. These combinations should not be used in animals with liver disease, blood dyscrasias, or a history of sulfonamide sensitivity. Precautions normally observed when animals are taking sulfonamides should be observed. The safety of ormetoprim–sulfadimethoxine has not been established in pregnant dogs, but studies indicate that the use of trimethoprim–sulfadiazine is safe in pregnant dogs. Trimethoprim–sulfonamides have caused thyroid disorders in piglets from treated sows. Trimethoprim is not absorbed following oral administration in ruminating animals.

Clinical Use

Dogs: Sulfadiazine combined with trimethoprim is used as a treatment for coccidiosis at 5–10 mg/kg BW trimethoprim combined with 25–50 mg/kg BW of sulfadiazine, given for 6 days to dogs over 4 kg BW. Dogs under 4 kg BW are given half this dosage for 6 days. Remission of clinical signs of hepatozoonosis can be obtained by oral administration of a combination of trimethoprim–sulfadiazine (15 mg/kg BW every 12 hours), clindamycin (10 mg/kg every 8 hours), and pyrimethamine (0.25 mg/kg every 12 hours) for 14 days (MacIntire et al., 2001). As the therapy is ineffective in eliminating the tissue stages of the organism, clinical response is short lived. Clinical relapses may be prevented by subsequent therapy with decoquinate (MacIntire et al., 2001).

Cats: Agents and dosages used for the treatment of canine coccidiosis are also used for the treatment of feline coccidiosis.

Ormetoprim

Ormetoprim combined with sulfadimethoxine (Rofenaid 40®) is the only combination DHFR/TS-sulfonamide presently available in the United States for poultry. Other combinations are intended for use in human beings, equines, and small animals. Ormetoprim is also available in combination with sulfadimethoxine in the ratio of 1 part ormetoprim plus 5 parts sulfadimethoxine (Primor®) in various size tablets.

Clinical Use

Chickens and turkeys: Combinations are fed to chickens at 0.0075% ormetoprim and 0.0125% sulfadimethoxine to prevent coccidiosis in broiler chickens. Do not feed to birds producing eggs for food. Ormetoprim combined with sulfadimethoxine is fed to turkeys at 0.00375% ormetoprim and 0.00625% sulfadimethoxine to prevent coccidiosis. Do not feed to birds producing eggs for food.

Dogs: Sulfadimethoxine combined with ormetoprim is used as a treatment for coccidiosis in dogs at 11 mg/kg BW ormetoprim plus 55 mg/kg BW sulfadimethoxine for up to 23 days.

Pyrimethamine

The advantage of pyrimethamine is that it has much greater affinity for the dihydrofolate reductase enzyme of protozoa than bacteria (Ferone et al., 1969). The inhibitory activity against protozoal dihydrofolate reductase was 140 times greater for pyrimethamine than trimethoprim; but by contrast, the activity against the bacterial enzyme was 500 times less for pyrimethamine than trimethoprim. Activity against mammalian blood cells is also greater for pyrimethamine, making this agent more prone to cause folate-deficient anemia in animals.

Pyrimethamine is available in 25-mg tablets. Pyrimethamine is well absorbed after oral administration and is slowly but extensively metabolized. Less than 3% is excreted in the urine in the first 24 hours, and the half-life is 4–6 days in human beings (Van Reken and Pearson, 1990). It accumulates in the kidneys, lungs, liver, and spleen. It is excreted in urine as metabolites, but some pyrimethamine can be found in the milk. Very high doses of pyrimethamine are teratogenic in laboratory animals. Pyrimethamine is available in tablets that contain 25 mg pyrimethamine and 500 mg sulfadoxine (Fansidar®), and in tablets that contain 12.5 mg pyrimethamine and 100 mg dapsone (Maloprim®). The equine formulation is a suspension (ReBalance®) that contains pyrimethamine (12.5 mg/ml) + sulfadiazine (250 mg/ml).

Clinical Use

Avian: Pyrimethamine (1 ppm) combined with sulfadimethoxine (10 ppm) will prevent but not cure *Leucocytozoon caulleryi* infections in birds.

Dogs and cats: For toxoplasmosis, cats and dogs are treated orally with 0.25–0.5 mg/kg BW pyrimethamine and 30 mg/kg BW sulfonamide (preferably, sulfadiazine) every 12 hours for 2–4 weeks. Canine neosporosis will also respond to the above regimens of DHFR/TS inhibitors and sulfonamides used to treat canine toxoplasmosis. Bone marrow suppression can occur with the synergistic use of pyrimethamine and sulfonamides and can be corrected by the addition of folinic acid (5 mg/day) or by the addition of yeast (100 mg/kg BW daily) to the animal's diet.

Horses: Treatment recommendations for equine protozoal myelitis include sulfadiazine at 20–30 mg/kg BW orally twice daily combined with oral pyrimethamine at 1 mg/kg BW once daily for 30 days after clinical signs have stopped improving. Twelve weeks is the average length of treatment time. Relapse is possible if horses are not treated long enough, and reactivation of the infection may occur during periods of unusual stress. This treatment has fallen out of favor, because of the length of treatment required; however, it provides a less expensive alternative compared to other treatments. The presence of food may adversely affect the absorption of DHFR/TS inhibitors, and food should be withheld 1–2 hours before and after treatment. Cell culture studies have shown that pyrimethamine kills developing *S. neurona* at 1.0 μg/ml and trimethoprim kills it at 5.0 μg/ml (Lindsay and Dubey, 1999). These levels of agent probably are not reached in the treated horses. It has been suggested that intermittent (every 2 or 4 weeks) single EPM treatment may help prevent clinical relapse in horses recovering from EPM (MacKay et al., 1992). If relapse does occur, the entire treatment regimen should be repeated. A study of 12 horses with EPM on a farm with an EPM outbreak indicated that combination treatment with trimethoprim–sulfonamides and pyrimethamine resulted in transient fever, anorexia, and depression in two; acute worsening of ataxia in two; mild anemia in four; and abortions in three (Fenger et al., 1997). Prolonged use of DHFR/TS inhibitors has long been known to result in bone marrow suppression. Therefore, horses should be examined periodically (about every 2 weeks) for anemia and leukopenia. If the neutrophil counts drop below 3000 cells/μl, treatment should be discontinued until the counts recover (MacKay et al., 1992). Caution should be used when treating pregnant mares with this combination of drugs, as congenital defects, including skin lesions, bone marrow aplasia, and renal dysplasia, have been reported (Toribio et al., 1998). This occurred despite folic acid supplementation to the mares during treatment. Folinic acid, not folic acid, is recommended for treatment of folate deficiency; however, it may reduce the efficacy of the DHFR/TS inhibitor as some protozoal species are able to utilize folinic acid (Piercy et al., 2002).

Lincosamides

Spectrum: Toxoplasmosis, *Neospora* sp., hepatozoonosis.

Drugs included: Clindamycin, Lincomycin (Figure 42.18).

Clindamycin

Clindamycin (Antirobe®, Cleocin®) has been used for treatment of disseminated toxoplasmosis in cats and dogs (Lindsay et al., 1997b). The pharmacology of lincosamides, including clindamycin, is discussed in more detail in Chapter 36. It is a semisynthetic compound produced by alteration of the parent molecule lincomycin, which is produced by *Streptomyces lincolnensis*. It differs from lincomycin in having a chlorine group at C-7 rather than a hydroxyl group. The dosages used against *T. gondii* are higher than for the treatment of the aerobic and anaerobic bacteria for which it has an approved indication. Clindamycin is active against tachyzoites of *Toxoplasma gondii* and is initially coccidiostatic but becomes coccidiocidal after a few days of treatment. Oral and parenteral formulations of clindamycin have similar activity. The drug is almost completely absorbed after oral administration in both dogs and cats, and peak serum concentrations occur within 75 minutes (Harari and Lincoln, 1989). The half-life is about 5 hours after oral or IV administration. Clindamycin is widely distributed in many tissues and fluids and crosses the placenta and blood–brain barrier. It is metabolized in the liver and excreted in the bile and urine as parent agent and metabolites.

Clinical Use

Dogs and cats: For toxoplasmosis, cats are treated with clindamycin at 10–12 mg/kg BW orally every 12 hours for 4 weeks or treated with 12.5–25 mg/kg BW clindamycin IM every 12 hours for 4 weeks.

Dogs are treated with 3–13 mg/kg BW orally or IM every 8 hours for 2 weeks or with 10–20 mg/kg BW orally or IM every 12 hours for 2 weeks. Clindamycin is also combined with pyrimethamine and used for the treatment of toxoplasmic encephalitis in human beings;

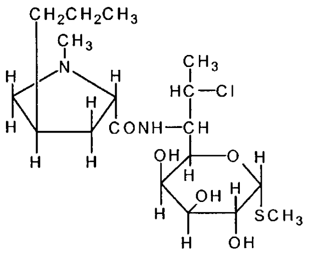
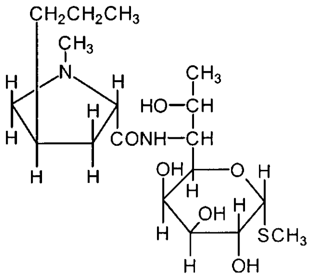
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Clindamycin	(2*S*-*trans*)-methyl-7-chloro-6,7,8-trideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]-amino]-1-thio-L-threo-α-D-galacto-octopyranoside ($C_{18}H_{33}ClN_2O_5S$) [424.98]	
Lincomycin methyl	6,8-dideoxy-6-[[(-1-methyl-4-propyl-2-pyrrolidinyl)carbonyl]amino]-1-thio-D-erythro-α-D-galacto-octopyranoside ($C_{18}H_{34}N_2O_6S$) [406.56]	

Figure 42.18 Lincosamides.

such combinations might have applications in veterinary medicine. Neonatal canine neosporosis is treated with 12.5–18.5 mg/kg BW clindamycin orally twice daily for 2–4 weeks (Dubey and Lindsay, 1998). Death may be prevented in pups with severe hindlimb involvement, but hindlimb function usually does not return to normal. The amount of clinical improvement will depend on the degree of paralysis that has occurred.

The response of *H. americanum*–infected dogs to treatment with a combination of trimethoprim plus sulfadiazine (orally 15 mg/kg BW every 12 hours) plus clindamycin (orally 10 mg/kg BW every 8 hours) plus pyrimethamine (orally 0.25 mg/kg BW daily) (TCP therapy) for 14 days demonstrated that remission of clinical signs in naturally infected dogs occurred in 2–3 days, but most treated dogs relapsed and eventually died from hepatozoonosis (Macintire et al., 2001). The best results have been seen in dogs that have undergone TCP therapy and then been placed on a daily oral treatment program with 10–20 mg/kg BW decoquinate. The decoquinate treatment must be continued because once it is stopped, relapse and clinical disease occur (Macintire et al., 2001). Dogs have tolerated this treatment and survived for over 18 months.

Azalides

Spectrum: Cryptosporidiosis, toxoplasmosis, babesiosis, cytauxzoonosis.

Drugs included: Azithromycin (Zithromax®).

The azalide antimicrobials are derivatives of the macrolides. The pharmacology of the macrolides is discussed in more detail in Chapter 36. They have activity against bacteria, as well as mycoplasma and have been investigated for the treatment of cryptosporidiosis in human patients with HIV infection, with some success (Kadappu et al., 2002). In vitro activity against *Toxoplasma gondii* has also been demonstrated. Azalides exhibit their antibacterial activity through inhibition of protein synthesis by binding to the 50S ribosomal unit of susceptible bacteria. The antiprotozoal mechanism of action is unknown.

Azithromycin

Azithromycin differs from other azalides and the macrolides by having a much higher volume of distribution and by its ability to accumulate within phagocytic cells. Concentrations are 100–200 times higher in neutrophils than in the plasma (Davis et al., 2002). The clinical effectiveness has been attributed to the high concentrations in cells and some tissues. However, it also has other immunomodulatory effects and may improve patients by exerting therapeutic benefits not completely explained by the antimicrobial activities (Parnham et al., 2014).

Clinical Use

Cattle: Azithromycin dihydrate given to calves naturally infected with cryptosporidiosis under field conditions, at doses of 1500 or 2000 mg per day, had a lower incidence of diarrhea, decreased oocyst shedding, and increased weight gains (Elitok et al., 2005; Nasir et al., 2013). The recommended treatment regimen is 1500 mg/calf/day for 7 days.

Dogs: Azithromycin (10 mg/kg PO q 24 h) administered concurrently with atovaquone (13.3 mg/kg PO q 8 h) for 10 consecutive days was shown to completely eliminate *Babesia gibsoni* infection from 8 of 10 dogs treated (Birkenheuer et al., 2004). The dogs in that study had previously been treated unsuccessfully with imidocarb or diminazene.

Cats: In cats diagnosed with *Cytauxzoon felis*, atovaquone (15 mg/kg PO q 8 h) administered concurrently with azithromycin (10 mg/kg PO q 24 h) for a duration of 10 days was shown to significantly increase survival compared to cats treated with imidocarb dipropionate (3.5 mg/kg given IM once and then repeated 7 days later) (Cohn et al., 2011).

4-Aminoquinolines

Spectrum: *Plasmodium* sp.

Drugs included: Chloroquine.

Chloroquine

Chloroquine has been used in the treatment of avian malaria. The mechanism of action against malarial organisms involves concentration of the drug, which is a weak base, within the acidic food vacuoles of the parasites. Once inside the cell, chloroquine causes rapid clumping of heme pigments by inhibiting plasmodial heme polymerase activity (Slater and Cerami, 1992). Heme then accumulates to toxic levels, causing cell death.

Clinical Use

Penguins: Clinical malaria in penguins that are kept in zoos or aquariums is caused by *Plasmodium relictum*. It is treated with an oral loading dose of 10 mg/kg BW chloroquine, followed by oral doses of 5 mg/kg BW given 6, 18, and 24 hours later (Clubb, 1986).

Diamidine Derivatives

Spectrum: Piroplasmosis, *Hepatozoon canis.*

Drugs included: *Aromatic derivatives:* diminazene diaceturate, pentamidine isethionate, phenamidine isethionate. *Carbanilide derivatives:* amicarbalide, imidocarb dipropionate (Figure 42.19).

These diamidine derivatives bind to DNA and interfere with parasite replication (Pilch et al., 1995; Patrick et al., 1997). This class of drugs has a tendency to accumulate in tissues; therefore, half-lives are very long, which may lead to residue problems in food-producing animals. Imidocarb was still detectable in liver samples from treated cattle 224 days after drug administration (Coldham et al., 1994). Specific drugs have been shown to cross the placenta, as well as the blood–brain barrier. Toxicity may involve renal disease, hepatic disease, or pulmonary congestion and edema, and is dose dependent. Treatment of babesiosis may produce clinical cures in some animals, but the infection is not completely cleared, leading to a carrier state in affected individuals. This depends on the susceptibility of the organism being treated.

Diminazene Diaceturate

Diminazene diaceturate (Berenil®, Ganaseg®) has a very long half-life, which is reported to be up to 29 days in the plasma of cattle, with the highest concentrations in tissues being found in the kidneys (Mdachi et al., 1995). Diminazene is excreted into the milk, with residues being reported for up to 6 days in sheep and goats (el Banna et al., 1999), and up to 21 days in cattle (Mdachi et al., 1995). Parasitic infections can alter the pharmacokinetics of this drug.

Clinical Use

Horses: *Babesia caballi* infections can be treated with 3–5 mg/kg BW IM, but 6–12 mg/kg BW is required for *B. equi* in horses. Doses as low as 0.5 mg/kg BW can be effective against *B. bigemina*. Diminazene at 5.0 mg/kg BW will eliminate *B. caballi* from horses if given twice during a 24-hour period (Kuttler, 1988).

Cattle: Diminazene at a dose of 3–5 mg/kg IM is effective against *B. bigemina* and *B. bovis* infections (Vial and Gorenflot, 2006). Doses of 3.5–7 mg/kg IM have been used for treatment of trypanosomiasis.

Dogs: A single 3.5–5 mg/kg BW IM treatment with diminazene is effective against canine babesiosis caused by *Babesia canis*, but 3.5 mg/kg BW given twice over 24 hours is required for *B. gibsoni*. The drug may enable survival against acute hemolytic crises and clinical improvement, but is inconsistent in clearing infection. Higher cumulative doses may produce central nervous system signs.

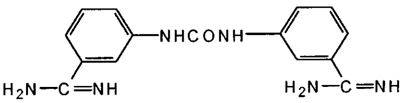
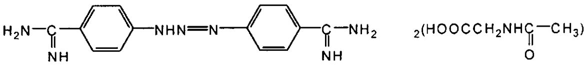
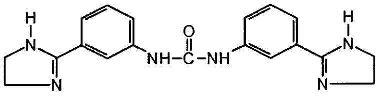
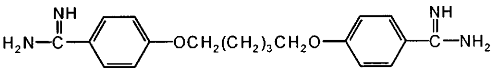
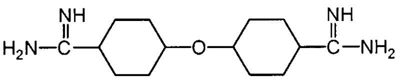
Name	Chemical name (Empirical formula) [Molecular weight]	Chemical structure
Amicarbalide	3,3′-(Carbonyldiimino)*bis*-benzenecarboximidamide ($C_{15}H_{16}N_6O$) [296.34]	
Diminazene	*N*-acetylglycine compounded with 4,4′-(1-triazene-1,3-diyl) *bis*-(benzenecarboximidamide) ($C_{22}H_{29}N_9O_6$) [515.54]	
Imidocarb	3,3′-di-2-imidazolin-2-yl-carbanilide ($C_{19}H_{20}N_6O$) [348.41]	
Pentamidine	4,4′-[1,5-pentanediylbis(oxy)] *bis*benzenecarboximidamide ($C_{19}H_{24}N_4O_2$) [340.43]	
Phenamidine	4,4′-oxybisbenzenecarboximidamide ($C_{14}H_{14}N_4O$) [254.29]	

Figure 42.19 Diamidine derivatives.

Cats: For treatment of *Cytauxzoon* infection, two injections are given intramuscularly at 2 mg/kg BW within a 1-week interval (Greene et al., 1999). Diminazene is ineffective for elimination of the carrier state of *Cytauxzoon* infection in cats (Lewis et al., 2014). Diminazene is not effective against *B. felis* but is effective against *B. herpailuri* in cats.

Pentamidine Isethionate

Pentamidine isethionate (Lomidine®, Pentam 300®) is useful in programs that are attempting to produce immunity in cattle by abbreviating infections.

Clinical Use

Cattle: When given SC during the acute phase at 0.5–2 mg/kg BW, pentamidine usually produces a clinical cure, but as much as 5 mg/kg BW will not completely eradicate the parasite from cattle.

Dogs: Pentamidine is effective against canine babesiosis when given IM at 16.5 mg/kg BW on 2 consecutive days but may cause adverse reactions, including pain at the injection site, hypotension, tachycardia, and vomiting. It has also been used in the treatment of leishmaniais at a dose of 4 mg/kg BW IM twice weekly for 4 weeks. This regimen was repeated again 3 weeks later. Clinical remission was obtained in all dogs treated, and signs did not appear during a 6-month follow-up period (Noli and Auxilia, 2005). Pentamidine isethionate given at 4 mg/kg BW intramuscularly at days 14, 16, 18, 20, 22, 24, and 26 following experimental infection constituted a safe and efficient trypanocide in *T. brucei brucei* infected dogs (Akpa et al., 2008).

Phenamidine Isethionate

Phenamidine isethionate (Lomadine®) can be used to treat bovine and equine *Babesia* infections with 8–13 mg/kg BW IM. Phenamidine at 8.8 mg/kg BW will eliminate *B. caballi* from horses if given twice during a 24-hour period (Kuttler, 1988).

Dogs: Phenamidine isethionate given SC at 15–20 mg/kg BW once a day for 2 days, is effective against canine babesiosis caused by *B. gibsoni*; a single dose of 8–13 mg/kg is effective against *Babesia canis*.

Amicarbalide

Amicarbalide (Diampron®) is effective against bovine babesiosis when given IM at 5–10 mg/kg BW. Amicarbalide at 8.8 mg/kg BW will eliminate *B. caballi* from horses if given twice during a 24-hour period but will not eliminate *B. equi* when used at levels of 22 mg/kg BW, which approaches toxic levels (Kuttler, 1988).

Imidocarb Dipropionate

Imidocarb diproprionate (Imizol®) is one of the treatments of choice for babesial infections. Also, administration of imidocarb may interfere with the efficacy of some babesial vaccines in certain species (Combrink et al., 2002). In cattle, the elimination half-life of imidocarb in liver was 48.5 days and 120 days in muscle (Coldham et al., 1995). A dose-dependent hepatic toxicity has been noted.

Clinical Use

Cattle: Imidocarb is effective against bovine babesiosis when given at 1–3 mg/kg BW IM or SC.

Horses: Imidocarb given at 1–2 mg/kg BW will eliminate *B. caballi* from horses if given twice during a 24-hour period (Kuttler, 1988). When given at 4 mg/kg IM every 72 hours for a total of four doses, imidocarb produced a significant increase in the urine γ-glutamyl transferase (GGT) : creatinine ratio, as well as mild azotemia in normal horses (Meyer et al., 2005). Liver function was not affected. This dose has been shown to be ineffective in eliminating the carrier state in *B. equi*–infected animals (Kuttler et al., 1987). At higher doses (16 and 32 mg/kg BW), renal failure, hepatic failure, and pulmonary edema were noted (Adams, 1981). When administered to pregnant mares, imidocarb was detectable in fetal blood at levels similar to those found in the dam's blood (Lewis et al., 1999). Imidocarb is also used in the treatment of *Theileria equi*; however, sensitivity varies up to fourfold depending on the strain (Hines et al., 2015) and may explain why some animals fail to completely clear the organism.

Dogs: A single 7.5 mg/kg BW SC imidocarb treatment will completely clear *Babesia canis* infections in dogs (Penzhorn et al., 1995). A single dose of 3.5 mg/kg BW SC diminazene, followed the next day by a single dose of 6 mg/kg BW SC imidocarb, will completely clear *Babesia canis* infections in dogs (Penzhorn et al., 1995). To eliminate *Babesia canis*, the recommendation when using imidocarb alone is 6.6 mg/kg IM or SC, and repeat in 14 days (Birkenheuer, 2014). Although imidocarb is not considered a first choice against *Babesia gibsoni* infections in dogs, it has produced clinical improvement (Birkenheuer, 2014). *Hepatozoon canis* infections are treated with 5–6 mg/kg BW SQ or IM every 2 weeks until gamonts are no longer present in blood smears (Baneth and Weigler, 1997). Complete elimination of *H. canis* with this protocol may frequently not be achievable (Sasanelli et al., 2010).

Cats: For treatment of *Cytauxzoon* infection, two injections are given intramuscularly at 3.5 to 5 mg/kg BW within a 1–2 week interval (Greene et al., 1999; Cohn et al., 2011). Control of parasympathetic side effects is recommended by pretreating cats with atropine or glycopyrrolate. Feline babesiosis is refractory to treatment with imidocarb.

Nitrothiazole Derivatives

Spectrum: *Giardia, Coccidia, cryptosporidiosis, S. neurona*

Drugs included: Nitazoxanide.

The antiprotozoal action of the nitrothiazole derivatives is thought to be via interference with the pyruvate-ferredoxin oxidoreductase enzyme dependent electron transfer reaction, which is essential for anaerobic energy metabolism of the parasites (Parashar and Arya, 2005). It is specific for parasites capable of intracellular reduction of the nitro group on the drug to a toxic-free radical. Nitazoxanide (NTZ) is rapidly converted to an active metabolite, deacetylnitazoxanide (also known as tizoxanide), in the horse (Navigator Paste, package insert), as well as in humans (Parashar and Arya, 2005).

Nitazoxanide

Nitazoxanide (Navigator®) was available as a 32% paste to treat equine protozoal myeloencephalitis (EPM). The adverse effects in horses are numerous and can be severe, including a fatal enterocolitis at recommended doses; therefore it was voluntarily withdrawn from the market by the sponsor. In vitro and in vivo efficacy against *Cryptosporidium* organisms has also been demonstrated (Jenkins, 2004). It is approved by the FDA as a treatment for cryptosporidial infection in children (Alinia®).

Clinical Use

Horses: Field trials with NTZ have shown a success rate, defined by an improvement in neurological signs and/or conversion to a negative result on western blot of the CSF, of 57–81%. This included a 78% success rate for horses that had previously been treated with other drugs for EPM. Horses are treated for 28 days. For the first 5 days, the dose is 11.36 mg/lb (25 mg/kg) orally once daily. The dose is then increased to 22.72 mg/lb (50 mg/kg) orally once daily for the remaining 23 days. This drug should not be used in horses less than 1 year of age or in those horses that have concurrent disease.

Cattle: Nitazoxanide was shown to reduce the duration of shedding of *Cryptosporidium parvum* oocysts and the severity of diarrhea in experimentally infected calves. For treatment of cryptosporidiosis, calves were orally administered 1.5 grams NTZ suspended in water twice a day (Ollivett et al., 2009).

Cats: Sustained inhibition of *Cryptosporidium* oocyst shedding in the feces of four naturally infected cats after a single oral dose of 75 mg/kg BW has been reported. However, reshedding of oocysts was prompted by administration of corticosteroids. NTZ is not well tolerated by cats and results in vomiting and dark, foul-smelling diarrhea (Gookin et al., 2001).

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43

Ectoparasiticides

Ronald E. Baynes

Introduction

The focus of this chapter is to describe the pharmacology of veterinary drugs and pesticides currently approved to control important ectoparasites that adversely affect domestic animal health and production. These ectoparasites are primarily insects (fleas, flies, lice) and ascarines (ticks and mites). Effective control of these ectoparasites is not only critically important for the veterinary patients, but also from a public health perspective as several of the animal ectoparasites not only transmit disease between animals but they can also transmit major diseases to humans. Fleas are known vectors for tapeworms, *Diplidium caninum,* and humans can develop cat scratch disease when *Bartonella henselae* is transmitted from cats to humans via cat fleas. Flea allergy dermatitis is major concern for companion animals. These are amongst the most important reasons why flea control in companion animals is critical, and explains why annual expenditures to control fleas have totaled in excess of $2.0 Billion in the USA and Western Europe (Kramer and Mencke, 2001). Ticks can transmit Lyme disease, Rocky Mountain spotted fever, babesia in dogs and cattle, and heartwater in ruminants, to mention only a few. Mosquitoes can transmit West Nile virus to humans and heartworm diseases to pets, malaria to birds, rodents, and primates, and they also serve as biological vectors of viral encephalitides in horses. Flies not only transmit disease and cause damage to hides and coat of pets, their nuisance can result in decreased feed intake in livestock and thus reduced timely weight gain, resulting in economic loss to livestock farmers. Face flies are known to transmit the causative agent (*Moraxella bovis*) of pink eye or infectious bovine keratoconjunctivitis in cattle. Control of myiasis-causing flies can be significantly improved with effective ectoparasiticides.

The *feeding behavior* of ectoparasites differ and this may be critical if the drug of choice is strictly a repellent or adulticide. Adult fleas usually engorge themselves with a blood meal from the host with 5 minutes to 1 hour of infestation of the animal, with female fleas feeding for about 25 minute and males feeding for 11 minutes (Cadiergues et al., 2000). In order to prevent flea allergy dermatitis, an effective ectoparasiticide should be able to prevent this feeding behavior. Comparative efficacy studies suggest that organophosphates (OPs) and/or pyrethroids are more effective than the newer ectoparasiticides in preventing the flea from taking the first blood meal (Franc and Cadiergues, 1998). Ticks feed on a blood meal for a longer period (days) before it is completed. Under these conditions pathogens such as *Borrelia burgdorferi,* which causes Lyme disease, become well adapted to this long feeding period. However, with other tick-borne pathogens, such as *Ehrlica* and *Rickettsia*, transmission can occur within the first hours. For these reasons, the use of combination drugs (insecticide and repellent) will prevent ticks from attaching and feeding (Young et al., 2003). A good example of this desired combined efficacy is a drug formulation containing the repellent permethrin and the acaricide imidacloprid.

The ideal ectoparasiticide should not only be an effective repellent and adulticide, it must also be *persistent* in the blood or skin surface at therapeutic concentrations for a significantly long time, such as 1–3 months, so that there is adequate client compliance. Another challenge is that the formulation of an insecticide must be *stable* to sunlight and also water/shampooing. An excellent example of this is imidacloprid, which is photostable and persistent after shampooing compared to the early generation pyrethroids that were not very stable in sunlight. Some formulations, such as Frontline Plus®, contain the adulticide (fipronil) as well as an insect growth inhibitor (methoprene), because by the time pets are observed to be infested with fleas, the fleas would already have laid eggs. A final consideration in choice of therapy is whether to *treat the environment* as well as the animal. There appears to be a new paradigm within the last 10 years of treating the pet and not the environment (Rust, 2005).

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Pharmaceutics of Topical Formulations

Many of the commercial topical ectoparasiticides used to treat domestic animals are formulated to be released from (i) liquid organic/aqueous formulations and/or (ii) synthetic polymers such as collars and ear tags. The rate of penetration through the skin or translocation across the skin surface is dependent on several critical pharmaceutical properties. Liquid formulations include pour-ons, topical sprays, and dips, which consist of the active ingredient and other (formerly called inert) ingredients that can modulate dermal pharmacokinetics. These other ingredients include mostly alcohols, oils, and spreading agents (Magnusson et al., 2001). The ear tags are composed of a solid organic polymeric macromolecule (e.g., polyvinyl chloride) and an active ingredient that are mixed with the tag and formed by injection molding. These devices are designed to slowly release the insecticide over a defined time period (e.g., Cutter Gold® last 5 months) as the animal moves its head, but it should be removed at the end of the fly season or before slaughter.

Transdermal Delivery

Topical application is the more common for administration of ectoparasiticides. An understanding of the dermal pharmacokinetics of these drug and pesticide formulations will further the understanding of drug efficacy and safety. The fundamentals of this topic are covered in Chapter 47. Many of these drugs and pesticides are very lipophilic with large molecular weights, and therefore it is not surprising that there is slow dermal absorption and low systemic *bioavailability*, as well as a large volume of distribution and long plasma and tissue half-lives. All of these are important factors when considering repeat applications and strategies to minimize the emergence of pest resistance, whether it is in a house, kennel, or pasture, and also to minimize drug residues in food-producing animals.

The advantages of transdermal delivery include ease of application to the animal's coat and it avoids the problems of first-pass metabolism and also the gastrointestinal degradation often associated with oral administration. Some of the major disadvantages associated with this route of administration are pet owners sometimes overdose the animal with topical sprays or pour-on formulations, which often result in toxicoses in sensitive populations such as feline species and juveniles. Licking is part of the natural grooming and social behavior of many animal species, which can result in significant oral absorption and subsequently increased systemic exposure in the treated and also untreated animals that are part of a large livestock herd (Laffont et al., 2003). Application of pour-on products to food animals can result in significantly longer withdrawal times when compared to administration by oral or injection routes. This is most evident with ivermectin pour-on products, which cause significant retention of ivermectin in the skin layers and thus prolong release of the drug. For example, this results in a 45-day withdrawal time for the pour-on product versus 35 days for the subcutaneous route (KuKanich et al., 2005).

Routes of Dermal Absorption

There are five possible routes (Figure 43.1) for a drug or pesticide to diffuse as it partitions from the formulation on the skin surface through the stratum corneum and viable epidermis before entering the microcapillaries at the epidermal–dermal juncture. Many, if not all, of the ectoparasiticides diffuse through the intercellular matrix (intercellular route) in the stratum corneum and viable epidermis. This matrix may vary biochemically between animal species, but in all species it consist of varying proportions of fatty acids, ceramides, and cholesterol. The reader should also be aware that apparent epidermal thickness (Monteiro-Riviere et al., 1990) varies across animal species in the following order, thereby influencing the intercellular path length for the diffusing pesticide:

Porcine	>	**Cattle**	>	**Canine**	>	**Feline**
(52 µm)		(37 µm)		(21 µm)		(13 µm)

The intracellular route and sweat pores are less likely routes for these lipophilic chemicals as they have more hydrophilic properties than the intercellular matrix.

Even though light microscopy examination of skin suggest that the hair follicle extends into the dermis and hypodermis, this is not really true. The hair follicle is

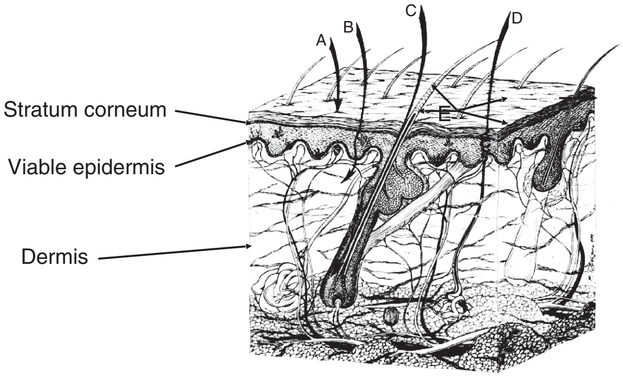


Figure 43.1 Pathways for drug and insecticide transport in mammalian skin. A, transcellular; B, intercellular; C, transfollicular; D, sweat pore; E, lateral diffusion.

really a deep invagination of the epidermis, and therefore if a drug travels down the hair follicle, it still has to cross the epidermis in order to gain access to the circulatory system. However, the presence of many hair follicles significantly increases the effective epidermal surface area, and thus increased flux, although the apparent surface area may be the same. This in part explains why care must be taken when administering pour-on ectoparasiticides in an extralabel manner across species. In essence, hair follicles and are arranged and distributed differently across animal species, and these differences can dictate transdermal delivery into the systemic circulation for pour-on products as well as translocation across the skin surface for topicals. The coat may contain primary and or secondary hair follicles. The former are large diameter and deep rooted into the dermis and associated with sebaceous and sweat glands. The secondary follicles are smaller in diameter, they are near the surface, and have sebaceous glands, but no sweat gland erector pili muscle. Horses and cattle have single hair follicles distributed evenly while pigs have single follicles grouped in clusters of four follicles. Dogs have primary and secondary follicles with as many as 15 hairs emerge from a single opening in the skin. Goats have primary follicles in groups of three, with three to six secondary follicles associated with each group. Sheep are bit more complex. They have hair-growing regions with mostly single follicles on face, distal legs region, and ears, and they have wool growing regions over most of their body, which can be defined as mostly compound follicles. A cluster will have three primary follicles and several secondary follicles.

Mechanisms of Action

Structures of many of the compounds to be discussed in this chapter are depicted in Table 43.1. Ectoparasiticides can have the following broad mechanisms of action that can affect the biology of the ectoparasite. More specific examples are summarized in Table 43.2.

- Ectoparasite nervous system
  - acetylcholinesterase (AChE) inhibitors
  - Na^+ channel blockers
  - nicotinic acetylcholine receptor (nAChR) inhibitors
  - γ amino butyric acid (GABA)/glutamate receptor Cl^- channel inhibitors
- Ectoparasite repellant
- Insect growth and development.

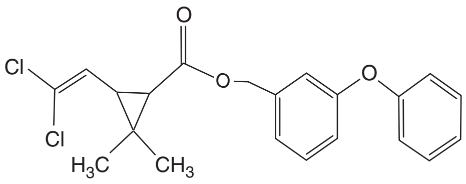
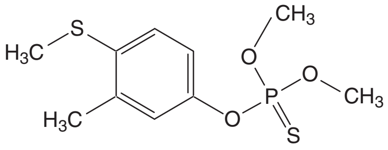
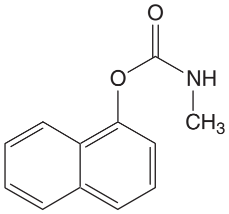
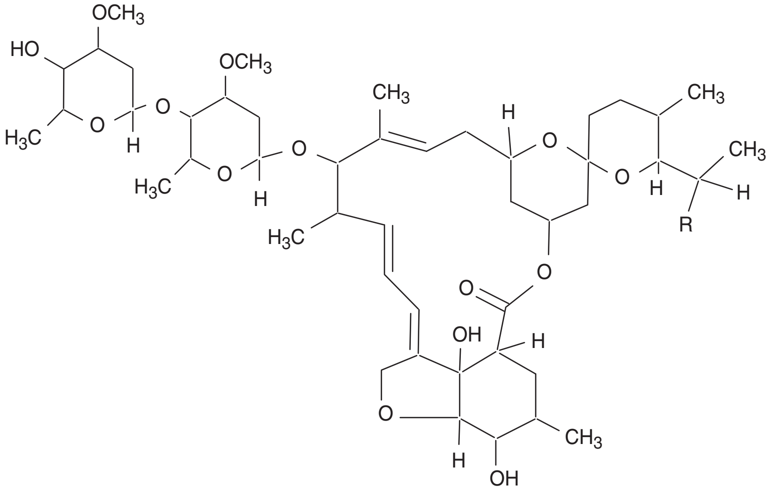
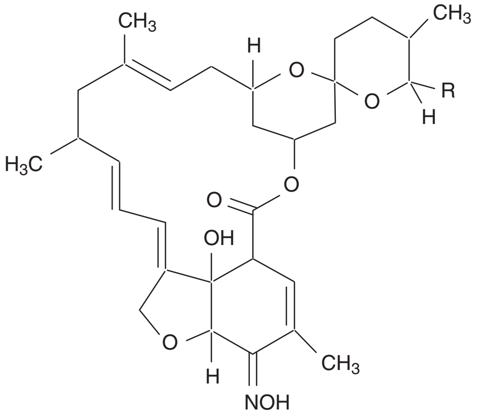
A good ectoparasiticide has a high efficacy against the ectoparasite and is not toxic to the host. Many of the OP and organochlorine pesticides are very effective and have a broad spectrum activity. However, because of their toxicity to mammals (Table 43.2), there are safer pesticides available for veterinary use, and fewer chemicals from these two classes are being used in veterinary medicine. For this reason, this chapter will not discuss the organochlorines especially as they are no longer approved for veterinary use. The newer ectoparasiticides such as the neonicotinoids (e.g., imidacloprid) are more selective for certain insects such as fleas, and very few other insects. However, the term broad spectrum may be misleading. Recall that ivermectin, which is often regarded as a broad-spectrum antiparasitic drug, is indeed highly effective against many nematodes and ectoparasites, but is without effect on cestodes and trematodes.

Resistance to Ectoparasiticides

Resistance has been widely reported for many popular ectoparasiticides such the avermectins, pyrethroids, and OPs. Resistance also tends to occur where there is intensive use of antiparasitic drugs, even on “well-managed” farms. There is some concern that as the newer insecticides such as the neonicotinoids become more frequently and inappropriately used, resistance can potentially emerge. However, there is little or no evidence of fleas developing resistance to the increasing and popular use of imidacloprid (Schroeder et al., 2003). Mechanisms of resistance may be related to decreased penetration, increased detoxification enzyme activity, and decreased sensitivity of the target site. These mechanisms have been more recently elucidated from a molecular basis using annotated insect genomes and recognizing that insect resistance is mediated by complex multigene enzyme systems. Pyrethroid resistance amongst insects has been associated with mutations in the sodium channel genes reducing the ability of the insecticide to bind to the sodium channel. Although resistance to imidacloprid has not been demonstrated in cat fleas, mutations in nicotinic acetylcholine receptors have been associated with some agronomic insects being resistant to this new class of insecticides (Liu et al., 2005).

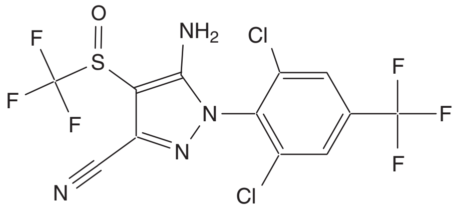
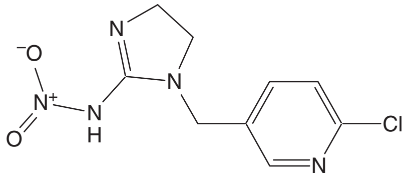
Cross resistance for a specific target site is indeed very common within individual insecticide classes. However, cross-resistance across different target sites is a more severe consequence and limits insect management. Detoxification mechanisms are often the source of cross-resistance between insecticides that differ in their mechanisms of action. These detoxification enzymes include the carboxylesterases, cytochrome P450s, and glutathione-*S*-transferases, which are often implicated in pyrethroid and OP resistance. In a more recent observation, point mutations in the *Rdl* gene in a field population of cat fleas conferred cyclodiene resistance and cross-resistance to fipronil, which is one of the few new chemicals on the commercial market (Daborn et al., 2004).

Table 43.1 Chemical structures and physicochemical properties of common ectoparasiticides

Name	Chemical name (empirical formula) [molecular weight] Log P	Chemical structure
Permethrin	3-(2,2-dichloroethenyl)-2,2-dimethyl-cyclopropane-carboxylic acid (3-phenoxyphenylmethyl) ester ($C_{21}H_{20}Cl_2O_3$) [391.29] Log P = 6.10	
Fenthion	Phosphorothioic acid O,O-dimethyl O-[3-methyl-4-(methylthio)phenyl] ester ($C_{10}H_{15}O_3PS_2$) [278.34] Log P = 4.84	
Carbaryl	1-naphthalenol methylcarbamate ($C_{12}H_{11}NO_2$) [201.22] Log P = 1.85	
Ivermectin	22,23-dihydroavermectin B_1 ($C_{48}H_{74}O_{14}$) [875.10]	
Milbemycin Oxime	5-didehydromilbemycin (oxime derivative) 80% A_4, 20% A_3 ($C_{31}H_{43}NO_7$) A_3 [541.68] ($C_{32}H_{45}NO_7$) A_4 [555.71]	

(*continued*)

Table 43.1 (*Continued*)

Name	Chemical name (empirical formula) [molecular weight] Log P	Chemical structure
Fipronil	5-amino-1-[2,6-dichloro-4-(trifluoro-methyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1H-pyrazole-3-carbonitrile ($C_{12}H_4Cl_2F_6N_4OS$) [437.15] Log P = 4.0	 O F S NH2 Cl F F N F F N F N Cl
Imidacloprid	1-[(6-chloro-3-pyridinyl)methyl]-4,5-dihydro-N-nitro-1H-imidazol-2-amine ($C_9H_{10}ClN_5O_2$) [255.66] Log P = 0.57	

Strategies that can prevent or delay resistance include: (i) appropriate selection of insecticides, (ii) insecticide rotations and mixtures, (iii) limited interactions with agricultural pesticides, and (iv) resistance monitoring. The latter is a major issue when it comes to treating fleas as there are no universally susceptible strains of cat flea known (Rust, 2005). However, the use of various genetic tools such as TaqMan-allele-specific amplification provide a promising method for assaying large populations of insects for insecticide resistance to the newer chemicals on the commercial market. With this in mind, the reader is encouraged to consult reviews (ffrench-Constant et al., 2004) on how the new genetic tools are addressing insecticide resistance.

Fipronil

Fipronil was first approved in 1996 for use in dogs and cats to treat for fleas and ticks. This chemical has been marketed as Frontline® Top Spot and Frontline® Plus for dogs, puppies, cats, and kittens, and Frontline® Spray Treatments for cats and dogs. The Plus product indicates that fipronil was formulated with methoprene, which is an insect growth regulator (IGR) (methoprene is discussed in more detail in Section Insect Growth Regulators). The TopSpot product contains 9.7% fipronil and the Spray contains 0.29%. A more recent fipronil formulation is Frontline® Tritak for dogs (9.8% fipronil, 8.8% methoprene, and 5.2% cyphenothrin) and Frontline® Tritak for

Table 43.2 Mechanisms of action of ectoparasiticides against the parasite and signs of mammalian toxicity

Drug	Mechanism of action	Signs of toxicity
Fipronil	Noncompetitively block passage of Cl ions through GABA- and glutamate-gated channels	Hyperactivity and convulsions related to GABA antagonism
Imidacloprid	Competitive inhibition at postsynaptic nicotinic acetylcholine receptors (nAChR) leads to influx of Na ions	Nicotinic effects (tremors)
Avermectins	Target glutamate-gated chloride channels	CNS depression and ataxia Target GABA- and glycine-gated chloride ion channels
Pyrethrins and pyrethroids	Target voltage-gated Na ion channels in axonal membrane	Type 1 syndrome (tremors, hyperexcitability) Type 2 syndrome (salivation, weakness)
Organophosphates and carbamates	Inhibition of acetylcholinesterase (AChE)	Muscarinic and nicotinic effects (miosis, tremors, depression)
Amitraz	Inhibits monamine oxidase	Activates α_2-adrenergic receptors (CNS depression)
Insect growth regulators	Falsely signals insect to remain immature stage Inhibition of chitin synthesis	No known toxic signs
Synergists	Inhibit cytochrome P450	Can potentiate drugs and pesticides

cats (9.8% fipronil, 11% methoprene, and 15% etofenprox). Cyphenothrin and etofenprox are pyrethroid or pyrethroid like and are discussed in Section Pyrethrins and Synthetic Pyrethroids.

Chemistry

This insecticide is classified as a phenylpyrazole with the molecular formula: $C_{12}H_4Cl_2F_6N_4OS$. Fipronil has a molecular weight of 437.15 and log P (n-octanol/water) of 4.0, and solubility of 2 mg/l in water and >10,000 mg/l in corn oil (Budavari, 1996). This high affinity for oils in the skin partly explains why dermal absorption is limited.

Pharmacokinetics

Topical application of the fipronil formulation between the shoulder blades is believed to result in surface translocation of the chemical over the entire body with significant deposition in sebum, sebaceous glands, and hair follicles. In effect, the sebaceous glands act as a reservoir, which continuously secretes firponil out of the hair follicle forming a layer on and in the stratum corneum and fur of the animal. Recognizing that 95% effective flea control requires 0.7 μg fipronil/g fur, as much as 4.5 μg/g and 1.6 μg/g have been detected in cats at 42 days and dogs at 56 days, respectively. Dermal absorption of ^{14}C-fipronil was less than 1% of the applied dose for all doses tested (0.88–48 mg/rat) and up to 24 hours exposure (Cheng, 1995).

Mechanism of Action

Fipronil acts on the central nervous system where it appears to be primarily effective in noncompetitively blocking the passage of chloride ions through GABA-gated and glutamate-gated chloride channels in ectoparasites. The latter mechanism exits in ectoparasites, but not mammals, suggesting that there are multiple insect target sites for this drug. A study has demonstrated that fipronil and its metabolite, fipronil sulfone, are potent inhibitors at these sites (Zhao et al., 2005). In mammals, fipronil binding to the GABA receptor is also less effective, which also accounts for its wide margin of safety. Fipronil sulfone has a higher potency than fipronil in mammalian GABA receptors and simultaneous exposure to other drugs that potentiate formation of the metabolite could potentially be toxic to mammals in these scenarios. However, data from the literature is conflicting as to whether metabolic conversion to the sulfone derivative represents detoxification. For example, pretreatment of mice with a cytochrome P450 oxidase inhibitor, piperonyl butoxide, increased the toxicity of fipronil by as much as sevenfold (Hainzl and Casida, 1996). Prior to its veterinary approval, fipronil was and is still approved for use in plant agriculture; however, photochemical conversion can result in a desulfinylated photoproduct that is 10-fold more potent than firponil at the mammalian chloride channel. Whether this will be concern for livestock that forage on exposed pastures is still to be determined.

Efficacy

Fipronil as a topical spray is effective against adult fleas, all stages of brown dog ticks, American dog tick, lone star ticks, and deer ticks. This product is approved for dogs, cats, and puppies or kittens (>8 weeks), and can provide protection for up to 30 days. The manufacturers claim that although the Topical Spot On product can be effective against fleas for as long as 3 months they still recommend that it be applied every month. Fipronil (9.8%) formulated with 8.8% *S*-methoprene (dogs) or 11% *S*-methoprene (cats) (Frontline® Plus), an insect growth regulator, is not only an effective adulticide, it also kills flea eggs and flea larvae. While excellent ectoparasiticidal efficacy against fleas, ticks, and chewing lice has been proven, there has been some concern about its repellent effects against ticks and also ticks already on the animal (Denny, 2001; Young et al., 2003). Compared to some pyrethroids and some OPs, fipronil is also not effective in preventing fleas from biting and feeding within the first hour of infestation and prior to being killed (Franc and Cadiergues, 1998). Firpoil is effective for the treatment and control of biting lice (*Trichodectes canis*) in canine infestations (Pollmeier et al., 2002, 2004). The label also claims that this drug can aid in the control of sarcoptic mange infestations. Note that Frontline® Tritak™ for dogs is different from that for cats in that different pyrethroids are used in the formulations and applicators should be aware of this difference (see discussion on pyrethroid esters and pyrethroid ethers later in Section Pyrethrins and Synthetic Pyrethroids).

Safety/Toxicity

Fipronil appears to have a wide margin of safety (rat dermal LD_{50} = 2000 mg/kg), and thus is regarded as a lower order of toxicity when used in veterinary applications. No adverse effects have been observed at five times the maximum dose rate in dogs and cats. However, slight skin or eye irritation have been observed after skin and eye exposure, respectively (Rhone Merieux, 1997). The oral and dermal LD_{50} in rats is 100 mg/kg and >2000 mg/kg, respectively (Budavari, 1996). Like the avermectins and organochlorines, fipronil's mechanism of action involves GABA receptor antagonism. At toxic doses, it can cause hyperactivity, hyperexcitability, and convulsions, which are correlated with increased spontaneous nerve activity and generation of prolonged high-frequency discharges with nerve stimulation. These symptoms are associated with blockage of the neuronal inhibition or membrane hyperpolarization because these antagonist are also blocking chloride uptake. This in effect results in stabilization of the GABA-bound form of the chloride channel (Bloomquist, 2003).

There appears to be no reported interactions between firponil and approved companion animal drugs and ectoparasiticides. Animals exposed to large doses of firponil via accidental oral absorption are expected to display seizures, and neurological and hepatic function should be monitored (Hovda and Hooser, 2002). Extralabel use in young or small rabbits can cause seizures and death. Multigenerational rat studies have concluded that this drug is not mutagenic or teratogenic.

Neonicotinoids

Imidacloprid

Imidacloprid has been approved for use in crop agriculture as a systemic insecticide as well as for treating adult fleas on dogs and cats. Amongst the many imidacloprid products, it is available as Advantage® (9.1% imidacloprid) for adult fleas and Advantage® II (9.1% imidacloprid and 0.46% pyriproxyfen) to target adult and early life stages of fleas. It is also formulated at 8.8% w/v with permethrin (44% w/v) in K9 Advantix® II to treat for fleas as well as ticks. The latter is approved for use in cats and not dogs because cats do not tolerate permethrin as well as dogs. Advantage Multi® (10% imidacloprid and 2.5% moxidectin for dogs/1.0% for cats) is a once a month application approved for dogs and cats to treat for adult fleas, intestinal worms, and prevent heartworm disease as well as treat sarcoptic mange in dogs and control ear mites in cats.

Chemistry

Imidacloprid belongs to a relatively new class of synthetic insecticides known as the neonicotinoids. Imidacloprid (chloronicotinyls nitroguanidine) more specifically belongs to the chloronicotinyls that were first synthesized in 1985. This chemical was found to have 62 to more then 3000-fold greater insecticidal activity than the nicotinoids (e.g., nicotine), and was introduced into the commercial market in 1991. These chemicals are photostable, which ensures prolong residual efficacy, and they are moderately soluble in water (0.51 g/l). The log octanol water partition coefficient of imidacloprid is 0.57 and it has a molecular weight of 255.7. Unlike the nicotinoids, the neonicotinoids are not ionized at physiological pH. Neonicotnoids therefore more readily penetrate the CNS of insects than nicotinoids because the former has a greater hydrophobicity. This physicochemical difference in physiological conditions partly explains why the neonicotinoids have greater insecticidal activity than the nicotine-based insecticides.

Pharmacokinetics

Topical application of imidacloprid to the skin does not result in significant dermal absorption into the blood stream, but rather surface translocation aided by body movement results in whole-body coverage. Its efficacy is dependent on contact with the flea at the surface of the animal's skin. Results from metabolism and toxicokinetics studies in other vertebrates suggest that if this chemical becomes systemic, then it is widely distributed to organs, but not to fatty tissues or the central nervous system (Sheets, 2001).

Mechanism of Action

At the nicotinic cholinergic synapse, acetylcholine (ACh) is normally released from the presynaptic membrane and by binding to the postsynaptic membrane this results in a conformational change in the receptor molecule that leads to activation and opening of the ion channel and subsequent influx of extracellular Na^+ and efflux of K^+. This results in change in membrane potential, and propagation of nerve impulse. Imidacloprid essentially mimics the effects of ACh by competitive inhibition at the postsynaptic nicotinic acetylcholine receptors (nAChR) and little or no effect on muscarinic acetylcholine receptors (Buckingham et al., 1997; Tomizawa and Casida, 2003). The former are widely and predominantly distributed in the neuropil regions of the CNS in insects, and imidacloprid is highly specific for nicotinic receptor subtypes unique to insects. However, instead of a depolarization of the postsynaptic neuron and transmission of the nerve impulse as normally seen with acetylcholine, imidacloprid causes a biphasic response. This consists of increased frequency of spontaneous discharge followed by a block in nerve pulse propagation along the neurons and then insect death (Schroeder and Flattum, 1984). Muscarinic receptors and other types of nicotinic receptors in mammals do not bind imidacloprid effectively, which accounts for its selectivity for insects (Kagabu, 1997). There is, therefore, no surprise that adverse effects are not observed when dogs were topically exposed to greater than the recommended dosage. Readers should be aware that although the neonicotinoids are selective, the nicotinoids are less selective and therefore more toxic to vertebrates than the neonicotinoids.

Efficacy

Compared to fipronil and selamectin, imidacloprid appears to be more readily taken up by the flea via body contact (Mehlhorn et al., 2001). This study demonstrated that imidacloprid killed larvae and adult fleas within 1 hour of exposure, while similar efficacy was achieved with the other two chemicals within 24 hours. It should be noted that none of these three chemicals had any repellent activity against fleas. Imidacloprid can kill 98–100% of adult fleas for at least 4 weeks on dogs and cats. This chemical has limited activity against ticks. Imidacloprid also stops fleas from biting within 3–5 minutes after application although the fleas do not die within this short time frame. Among the product claims for Advantage II® is the ability to kill fleas within 12 hours of application. This clearly is useful for preventing flea allergy dermatitis. Earlier studies demonstrated that shampooing or water exposure does not significantly reduce the efficacy of imidacloprid for up to 28 days (Cunningham et al., 1997).

In fall 2002, Bayer Corp. received registration from EPA for a topical ectoparasite K9 Advantix™, which is a topical insecticide formulated with imidacloprid and permethrin to repel and kill mosquitoes, ticks, and fleas on adult dogs and puppies 7 weeks of age and older for up to 4 weeks. The product's efficacy is not affected by swimming or bathing activities. Imidacloprid acts on the postsynaptic nicotinic receptor by "locking" it in the open position thus hyperstimulating the neuron, and permethrin keeps open the Na^+ ion exchange channels causing constant nerve impulses. Recall, that these would normally close after transmitting an impulse. There is, therefore, a synergistic interaction on the nervous system that is associated with an overwhelmed parasite nervous system, followed by rapid paralysis and death of the parasite. Due to their unique physiology and inability to metabolize certain compounds such as the pyrethroid (reduced glucuronidation activity in cats), this product must *not be given to cats.*

Safety/Toxicity

Clinical signs of toxicity are usually associated with nicotinic effects (e.g., tremors) and hepatic effects when dogs were exposed to a high dose of 41 mg/kg in the diet (Hovda and Hooser, 2002). However, except for one single reported case in cats (Godfrey, 1999),there are no published reports of imidocloprid causing systemic toxicity in domestic animal species following dermal exposure. The chemical does not appear to cause dermal irritation nor does it appear to cause dermal sensitization, although there are very rare anecdotal reports of adverse skin reactions. Imidacloprid does not appear to be a teratogen, reproductive toxicant, mutagen, or carcinogen (Sheets, 2001). The available products should not be given to puppies or kittens younger than 4 months.

Nitenpyram

Nitenpyram (Capstar™) is in the same class as imidacloprid (i.e., chloronicotinyl), and therefore has the same mechanism of action. This is, however, not a topical application, but an *oral adulticide* that is used against fleas in dogs and cats.

Chemistry

Nitenpyram, like imidacloprid, belongs to the new class of synthetic insecticides known as the neonicotinoids. Nitenpyram has a molecular weight of 270.72, a log octanol water partition coefficient of −0.64, and water solubility of 840 g/l.

Pharmacokinetics

Nitenpyram is readily absorbed (bioavailability = 100%) in the gastrointestinal tract with the time to maximum blood concentration being 1.21 hours in dogs and 0.63 hours in cats. The C_{max} is about 4.3 to 4.8 ppm in both dogs and cats, which is significantly greater than the blood concentration of 0.5–1.0 ppm nitenpyram required to kill 100% of the fleas. The half-life in dogs and cats is 2.8 hours and 7.7 hours, respectively, and it is primarily excreted in the urine.

Efficacy

The recommended minimum oral daily dosage is 1 mg/kg in tablet form with or without food and as often as once per day when fleas are seen on the animal. One dosage form (11 mg) is for cats and dogs up to 11 kg, and the other dosage form (57 mg) is for dogs between 11 and 57 kg. This chemical is also very effective against fleas that have become resistant to fipronil (Schenker et al., 2001). Based on the above pharmacokinetics, it is no surprise that fleas begin falling off of the coat of dogs and cats within 0.5 hours of administration of this drug (Rust et al., 2003; Rust, 2005). Efficacy reaches 98.6% in dogs and 98.4% in cats within 6 hours.

Safety/Toxicity

The acute oral LD_{50} in male rats is 1680 mg/kg, which suggest that this chemical is very safe. Ten times the recommended dose did not appear to cause adverse health effects in 7 to 8-month-old cats and 7-month-old dogs (Witte and Luempert, 2001). In the same study, administration of one, three, or five times the recommended daily dose for 6 months resulted in no adverse health effects in dogs and cats that were initially 8 weeks old. The drug is well tolerated for daily doses in puppies and kittens that are >4 weeks. No adverse reactions were observed when used with most other drugs or ectoparasiticides such as topical, carbaryl, fipronil, pyrethin, or even imidacloprid, which has a similar mechanism of action (Witte and Luempert, 2001). A transient itching period observed in dogs and cats within 1-2 hours after treatment with this product appears not to be associated with the chemical irritation, but attributed to fleas displaying increased trembling activity before dying and falling off the animal (Mahoney et al., 2001).

Spinosad

This neonicotinoid, which is a mixture of spinosyn A and spinosyn B (17 : 3 ratio), was approved in 2007 for once a month use in dogs, and in 2012 for cats (e.g., COMFORTIS® Chewable Tablets). Spinosad at 30 mg/kg dose is also formulated with milbemycin oxime (0.5 mg/kg) as TRIFEXIS® was introduced in 2011 for use in dogs only to prevent heartworm, treatment and control of fleas, and adult hookworm, roundworm, and whip worm. It has been used in sprays, dips, and pour-ons for sheep to control lice and blowfly strike in some countries. It is a contact and systemic insecticide and acaricide. Spinosad is quickly absorbed to blood after oral absorption. Oral bioavailability is greater than 70%, especially when administered with food, resulting in maximum plasma levels at 2 to 4 hours after administration. The mechanism of action is associated with activation of nicotinic acetylcholine receptors of the nerve cell membranes of insects that are different subunit targets from those of other known neonicotinoids. This limits the possibility for cross-resistance with similar related insecticides.

Safety/Toxicity

Adverse reactions are rare in dogs and cats and vomiting may be observed on the day of, or the day after, administration. The oral LD_{50}, in rats is 3600 mg/kg, suggesting that spinosad is very safe in spite of anecdotal reports of adverse effects of Triflexis® in 2014 that appeared to be unrelated to the active ingredients of this products but point to other unrelated factors. It should, however, be noted that there is the possibility for drug–drug interactions between spinosad and ivermectin as spinosad could inhibit the drug transporter P-glycoprotein (P-GP) which is also a substrate for ivermectin (Schricky, 2014). This finding may conflict with results from another study where 20 adult Collies homozygous or heterozygous for the *MDR1* gene mutation were dosed with combinations of spinosad and milbemycin at five time and 10 times the label dose, respectively, and there were no observed adverse effects related to these chemicals such as depression, ataxia, mydriasis, and hypersalivation (Sherman et al., 2010). In spite of these observations, the manufacturer has reported on its label that its postapproval experience has seen these adverse effects associated with concomitant use of extralabel ivermectin and not with the label use of ivermectin.

Dinotefuran

This neonicotinoid is approved for dogs and cats and has the same mechanism of action against fleas as described for the previously described neonicotinoids. One product, Vectra for Dogs™, which contains 22% dinotefuran and 3% pyriproxyfen, claims to provide effective monthly control of adult fleas, flea eggs, flea larvae, and flea pupae. Pyriproxyfen is a juvenile-hormone analog, which controls the immature stages of the flea.

Isoxazolines

The isoxazoline class of antiparasiticides have both insecticidal and acaricidal activities. Afoxolaner (NexGard®) is an isoxazoline that was first introduced in 2000 and in 2013 as a veterinary product with a labeled dose of 2.5 mg/kg to treat for fleas and ticks in dogs as a chewing tablet. The more recent isoxazoline, fluralaner (Bravecto®), was approved in 2014 by US FDA at a label dose of 25 mg/kg as a chewable tablet to control flea and ticks. Both of these chemicals are very lipophilic. Afoxolaner has a molecular weight of 625.87 and log octanol water partition coefficient of 6.7, while fluralaner has a molecular weight of 556.29 and a log octanol water partition coefficient of 5.35.

Pharmacokinetics

Following oral administration of 2.5 mg/kg afoxalaner as chewable tablet, the absolute bioavailability was 74% with a mean maximum plasma concentration (C_{max}) of 1.65 ± 0.33 μg/ml at 2–6 hours (T_{max}) (Letendre et al., 2014). In another study, liquid formulations of afoxalaner dosed at 2.5 mg/kg resulted in highest C_{max} value of 0.82 μg/ml and lowest C_{min} value of 0.09 μg/ml at 30 days postdose (Shoop et al., 2014). In vitro and in vivo assays determined that 0.16 μg/ml provided 100% control at 24 hours and Letendre et al. (2014) reported that the EC_{90} for fleas (*Ctenocephalides felis*) and ticks (*Rhipicephalus sanguineus* and *Dermacentor variabilis*) to be 0.023 μg/ml and >0.1 μg/ml, respectively. Afoxalaner appears to be widely distributed (volume of distribution = 2.68 ± 0.55 l/kg) in the body of the dog, although plasma protein binding is high (99.9%). This drug has a low systemic clearance (4.95 ± 1.20 ml/h/kg). Ultimately, the terminal plasma half-life is long in dogs (approximately 2 weeks), although there can be breed differences; the half-life of afoxalaner in Collies can be as long as 47 days. The chemical is metabolized in the liver (low extraction ratio) with metabolites and parent being eliminated via urinary and biliary excretion, with the latter being more important than the former and limited evidence of enterohepatic recycling.

The pharmacokinetics for fluralaner in dogs was very similar to that for afoxalaner. Oral and intravenous studies reported a low systemic clearance (5.83 ml/kg/h), large volume of distribution (3.2 l/kg) in spite of it high plasma protein binding, and long apparent half-lives from 12–15 days (Kilp et al., 2014). Oral bioavailability ranged from 20 to 34% and the drug plasma concentration was >0.01 μg/ml for the high dose of 50/kg at

112 days posttreatment No dogs showed any signs of toxicity following oral doses of 12.5, 25, or 50 mg/kg.

Mechanism of Action

Isoxazolines inhibit GABA-gated chloride ion channel inhibitors with nanomolar potency (Shoop et al., 2014). Glutamate-gated chloride ion channel receptors have also been implicated (Woods et al., 2011). This results in an irreversible hyperexcitation in targeted insects and ticks. Although GABA-gated chloride ion channels are the target for many other ectoparasiticides on the market (e.g., avermectins, fipronil, and cyclodiens), the isoxazolines bind to a different site and, furthermore, the avermectins activate rather than block these ion channels. It should be noted that the isoxazolines are more selective for the GABA receptor in insects and tick than those receptors in mammals.

Efficacy

The recommended minimum oral daily dosage for afoxolaner is 2.5 mg/kg in tablet form once a month. This chemical, as expected from the pharmacokinetics, is very effective against fleas (*Ctenocephalides felis* and *C. canis*) and ticks (*Dermacentor reticulatus, Ixodes ricinus, Rhipicephalus sanguineus*). Fleas will be exposed to the chemical following attachment and feeding and the onset of effect can occur within 8 hours of attachment; for ticks, the onset of death is within 48 hours of attachment. Fluralaner (Bravecto) should be administered orally as a single dose every 12 weeks. It quickly kills fleas and the tick species such as *Ixodes, Dermacentor*, and *Rhipicephalus* within 12 hours and also kills *Amblyomma americanum* (lone star tick) if the drug is administered every 8 weeks instead of 12 weeks.

Safety/Toxicity

As indicated above, these drugs are very selective for fleas and tick nervous system compared to mammalian nervous system. No adverse effects have been observed in dogs receiving doses five times the label dose. Adverse reaction in dogs have been rare thus far, although vomiting, diarrhea, lethargy, and anorexia have been observed. These products have not been evaluated in pregnant, breeding, or lactating dogs, therefore caution should be exercised when administering these dogs.

Macrocyclic Lactones

These macrolides consist of two major groups, avermectins and milbemycins, which are effective against many ectoparasites, as summarized in Table 43.3. The avermectins include ivermectin, eprinomectin, doramectin, and selamectin and the milbemycins include moxidectin and milbemycin oxime. These compounds are also discussed in Chapter 41.

Table 43.3 US FDA approved ectoparasiticides[a]

Drug class	Approved species	Ectoparasite approved indications
Avermectins/milbemycins		
Ivermectin	Cats	Ear mites
	Beef cattle	Lice, mites, ticks, grubs
	Pigs	Lice, mites
Doramectin	Beef cattle	Lice, mites, ticks, grubs
	Pigs	Lice, mites
Pour-on eprinomectin	Dairy and beef cattle	Lice, mites, flies, grubs
Injectable eprinomectin	Beef only	Lice, mites, flies, grubs
Selamectin	Dogs	Fleas, mites, ticks
	Cats	Fleas, mites
Moxidectin	Dairy and beef cattle	Lice, mites, flies, grubs
Milbemycin	Cats	Ear mites
Organophosphates		
Fenthion	Beef cattle	Grubs, lice, flies
Famphur	Beef cattle	Grubs, lice, flies, ticks
Amitraz	Dogs	Mites
	Dairy and beef cattle	Lice, mites, ticks
	Swine	Louse, mites
Nitenpyram	Dogs and cats	Fleas
Lufenuron	Dogs and cats	Fleas

[a] Approved as of March 2015.

Chemistry

Both avermectin and milbemycin groups are 16-membered macrocyclic lactones. The avermectins are products of *Streptomyces avermitilis* that have the 16-membered macrocycle replaced by a spiroketal unit at C-17 to C-28, a hexahydrobenzofuran at C-2 to C-8a, and a bisoleadrosyloxy disaccharide at C-13. The milbemycins are also products of *Streptomyces* and are structurally similar to the avermectins, but *lack the sugars at C-13.* The latter group is believed to enhance antiectoparasite activity. The nemadectins are classified as milbemycins, but differ from the milbemycins proper because they have a distributed double bond at C-26. An important nemadectin is moxidectin (e.g., Cydectin)**.** Eprinomectin (4″-epi-acetylamino-4″-deoxy-avermectin B1) is a true second-generation macrocyclic lactone synthesized from avermectin. Doramectin differs from ivermectin in that a six-carbon ring replaces a three- or four-carbon chain at the C-25 position of the basic ivermectin. Compared to ivermectin, these molecular differences result in a more lipophilic and persistent doramectin molecule.

Pharmacokinetics

The oral, IV, and SQ pharmacokinetics have been described in Chapter 41 of this book. The focus here will be to compare the dermatopharmacokinetics of topically applied avermectins. It should be first noted that eprinomectin and moxidectin pour-ons are formulated in >80% oil content. This encourages more drug depot formation on skin surface and stratum corneum, and thus *reduced bioavailability* compared to pour-on products with ivermectin and doramectin. The latter are formulated with isopropyl alcohol, which is more likely than oil to promote epidermal flux. Because of the high oil content of the eprinomectin and moxidectin formulations, there is evidence that it is water-fast and thus tolerant to rain and other forms of weather precipitation (Gogolewski et al., 1997).

The dermal bioavailability of doramectin is about 40% greater than ivermectin in cattle (Gayrard et al., 1999). The dermal bioavailability for doramectin pour-on is approximately 16% when compared to the subcutaneous route of administration. Following topical application of selamectin, this drug is slowly absorbed (flip-flop kinetics) into the blood stream and redistributed to form reservoirs in sebaceous glands as well as the hair follicle and basal cells of the epidermis (Sarasola et al., 2002). Dermal bioavailability appears to be significantly greater in cats (74%) than in dogs (4.4%), although this may be attributed to greater self-grooming in cats than in dogs. However, selamectin flux is three times greater in cat skin (0.1 $\mu g/cm^2/h$) than in dog skin (0.03 $\mu g/cm^2/h$). The half-life of this drug is greater in cats (69 hours) than in dogs (14 hours), and, in general, selamectin half-life is greater in cats and dogs following dermal > oral > IV administration. This demonstrates its persistence in the stratum corneum and upper epidermis. As with many of the avermectins, the dermal absorption process is the rate-limiting step, and the increased persistence in the body after dermal application should reduce reinfection rates by ectoparasites. There is also conflicting evidence of differences in selamectin disposition between male and female dogs (Sarasola et al., 2002; Dupuy et al., 2004).

Mechanism of action

In invertebrates, the avermectins primarily target the glutamate-gated chloride ion channels, but which are close in proximity to GABA-gated sites. It is possible that these endectocides may also potentiate GABA-gated sites. Chloride influx lowers cell membrane resistance and causes hyperpolarization of the resting potential of postsynaptic cells. Transmission to muscles is therefore prevented. The ectoparasite therefore displays ataxia, paralysis, and death. In contrast to GABA antagonists (e.g., lindane and fipronil) that lead to convulsions, the avermectins cause a different neurological syndrome that is characterized at first by a coarse tremor followed by ataxia and coma-like sedation. It should be noted that the avermectins can block GABA at low concentrations, but at high concentrations cause irreversible channel activation or enhancement of GABA effect, which is associated with at the classical signs of ataxia and sedation. These endectocides have a wide margin of safety because they are very selective for the glutamate-gated channels in nematodes and arthropods, but which are lacking in mammals. In mammals, the avermectins primarily target the GABA-gated, and possibly the glycine-gated, chloride channels (Bloomquist, 2003). Furthermore, in mammals, GABA-mediated neurotransmission occurs in the CNS, where the blood–brain barrier prevents uptake of the endectocides. These endectocides are also thought to interfere with parasite reproduction. It should be noted that unlike the macrolide antibiotics, these endectocides lack antibacterial and antifungal activity.

Efficacy

Ivermectin

Injectable formulations are used to control lice (*Haematopinus suis*) and mange mites (*Sarcoptes scabiei var. suis*) in swine and horn flies (*Haematobia irritans*), grubs (*Hypoderma bovis*), biting and sucking lice (*Lignathus vituli, Haemaotopinus eurystemus*), and mites (*Sarcoptes scabei var. bovis*) in cattle. Pour-on and oral formulations are also available for cattle. There is *no* immediate death to ticks in cattle, but reduced reproductive potential. As these drugs are eliminated in the feces, they are effective against dung-breeding flies. Not all commercial formulations of ivermectin are approved for the treatment of the most common type of cattle mange caused by chorioptic mite. In dogs**,** SC doses can be effective against otodectic, sarcoptic, and notoedric mange and control of demodectic mange. In cats a relatively new topical product (Acarexx®) is approved for treating adult ear mites (*Otodectis cynotis*) in cats and kittens 4 weeks of age and older. There is evidence that the ears do not necessarily need to be cleaned before treatment.

Eprinomectin

This drug is available as a pour-on formulation (Eprinex®) at 0.5 mg/kg for both dairy and beef cattle and it is also available as an extended-release subcutaneous injection (LongRange®) at 1.0 mg/kg for beef cattle only. The correlation between pharmacokinetics of macrocyclic lactones and the persistence of the antiparasitic activity has been a difficult exercise, but several investigators have estimated that minimal drug plasma concentrations between 0.5 and 1 ng/ml should be adequate for nematocidal activity, while others have suggested minimum levels of 1 to 2 ng/ml (Shoop et al., 1996; Lifschitz et al., 1999, 2008). The eprinomectin pour-on formulation resulted in plasma concentrations above 0.5 ng/ml for at least 21 days (Alvinerie et al., 1999) while the extended-release injectable formulation maintained plasma concentrations above this level for at least 150 days.

The pour-on formulation provides approximately 20–50 days of parasite control, depending on the species, while the injectable formulation provides up to 100–150 days of parasite control. The pour-on formulation is an oil-based formulation that makes the drug "waterproof" to some extent. This broad-spectrum antiparasiticide is effective against lice, hornflies, and mites. Because of its unique molecular structure and route of application, the pour-on eprinomectin at the label dose does not readily partition into milk. The milk/plasma ratio can range from 0.07 to 0.17 (Shoop et al., 1996; Alvinerie et al., 1999), and therefore is the one of few pour-on endectocides that requires *no* milk withholding time. The meat withdrawal time is also zero for the pour-on formulation. However, the injectable eprinomectin formulation, LongRange®, is only approved for beef cattle and not dairy cattle and the meat withdrawal time is 48 days.

Doramectin

Doramectin is available as an injectable for beef cattle (0.2 mg/kg SC and IM) and swine (0.3 mg/kg IM) and pour-on (0.5 mg/kg) for beef cattle only. It is believed to be more efficacious than other endectocides against biting and sucking lice. This drug is not approved for dairy cattle because of the concern for significant residues in milk. In cattle, the spectrum of activity is similar to that of ivermectin. Doramectin has activity against important sucking lice, grubs, ticks, mites, and screwworms in cattle. The latter is unique among the macrolide endectocides. Application of the pour-on can prevent reinfestation of lice for as long as 16 weeks. In swine, doramectin is approved for use against sucking lice and mange mites.

Moxidectin

Moxidectin products (Cydectin®, Equest®) are a new generation of milbemycins that have a broad spectrum of activity against nematode and arthropod parasites in cattle. Unlike the previously discussed endectocides, this is a single compound, and not a mixture of two compounds. The approved dose of Cydectin® pour-on is 0.5 mg/kg in cattle, and the drug has a spectrum of activity similar to ivermectin. Cydectin® *is the only pour-on endectocide labeled for control of common scab mite* (*Psoroptes ovis*), a reportable disease requiring quarantine.

Selamectin

Selamectin (Revolution®) is a topical monthly application approved for treating dogs and cats for ear mites, adult fleas, and prevents flea eggs from hatching. In dogs, it is used in the treatment and control of sarcoptic mange (*Sarcoptes scabiei*) and control of the tick (*Dermacentor variabilis*). Selamectin is the only spot-on product approved for control of canine sarcoptic mange. There are anecdotal reports of delayed responses to this drug and a few treatment failures (Curtis, 2004). However, in lieu of these situations, some veterinary dermatologist recommend that this drug be reapplied every 2–3 weeks with owners consent as this is extralabel use of the drug.

Milbemycin oxime

Milbemycin oxime is well known as a heart worm preventative, but it also has significant ectoparasiticidal effects. The only approved use of this chemical as an ectoparasiticide, Milbemite® Otic Solution, at 0.1% is used as a treatment for ear mite (*Otodectes cynotis*) infestations in cats and kittens 4 weeks of age and older. *Milbemycin oxime* may also be effective in cases of amitraz-resistant demodicosis, but this is not an approved use thus far.

Safety/Toxicity

Ivermectin has a 10-fold safety margin in ruminants, horses, swine, and dogs, except Collies. Due to a mutation within the gene that codes for the MDR1 pump protein that normally disallows entry of ivermectin into the central nervous system, Collies should not be treated with ivermectin or any other avermectin. Acute toxic signs include CNS depression, ataxia, and possible death. The principle clinical pathological change is a decrease in serum iron values in dogs.

The meat and milk withdrawal times for this class of drugs vary across avermectins, and Table 43.4 provides clear evidence of this and why label instructions should be carefully followed. Extralabel use of doramectin or ivermectin in dairy cattle may require a milk withholding period of almost 60 days!

Table 43.4 Withdrawal times of pour-on macrolide endectocides

Generic drug	Example	Approved species	Withdrawal times
Ivermectin	Ivomec®	Beef cattle	48 days for meat
Eprinomectin	Eprinex®	Beef and dairy cattle	0 days for meat and milk
Doramectin	Dectomax®	Beef cattle	45 days for meat
Moxidectin	Cydectin®	Beef and dairy cattle	0 days for meat and milk

Pyrethrins and Synthetic Pyrethroids

The pyrethrins were derived from *Chrysanthemum* plants, while in recent years they have been slowly replaced with synthetic pyrethroids. The latter are more resistant to breakdown, which results in greater residual activity than the pyrethrins. Pyrethrins and pyrethroids are often formulated with insect growth regulators, synergists, and/or repellents to enhance efficacy. The more frequently used in veterinary formulations pyrethroids include permethrin, fenvalerate, cypermethrin, and phenothrin. These may be formulated as pour-ons, spray-ons, barn misters, feed-throughs, and shampoos.

Chemistry

Natural pyrethrins occur as six compounds: pyrethrin I, pyrethrin II, jasmolin I, jasmolin II, cinerin I, and cinerin II (Kaneko and Miyamoto, 2001). The molecular weight of these insecticides range from 316.4 to 374.5 and log Ko/w range from 4.3 to 5.9. The synthetic pyrethroids are structurally similar to the natural pyrethrins, and many of those used in veterinary medicine have a wider range in molecular weights (302.4 to 416.3), log Ko/w (4.6 to 7.4), and LD_{50} (105.8 to 4240 mg/kg). Depending on the literature reviewed, the pyrethroids can be classified in a number of ways. Some authors have grouped them into two broad generations of pyrethroids, with the first generation being characterized as esters of chrysanthemic acid and alcohols having furan ring and terminal side chain moieties. These pyrethroids are sensitive to light and temperature, and are therefore most effective when used indoors. The second-generation pyrethroids, and for that matter the majority of pyrethroids in veterinary use today, have a 3-phenoxybenzyl alcohol in the alcohol moiety and replacement of the photolabile moieties with dichlorovinyl, dibromovinyl substituent, and aromatic rings. These modifications provide excellent insecticidal activity and environmental stability for this class of pyrethroids.

Another classification system groups the pyrethroids into four generations, with the first generation reflecting the same properties as the first generation in the earlier classification scheme. The first generation (e.g., allethrin) are similar to the natural pyrethrin, cinerin I, and therefore lack environmental stability. The second generation (e.g., resmethrin and tetramethrin) are significantly more stable than the first generation and have significantly greater killing capacity than the natural pyrethrins. The third generation include the ever popular pyrethroids, fenvalerate, and permethrin, which are noted for their photostability and potency. Permethrin in particular is the most ubiquitous pyrethroid formulated with veterinary ectoparasiticidal products. The fourth generation are the most potent of the pyrethroids (e.g., cypermethrin and cyfluthrin) and also the most persistent. For this reason they are most often formulated in ear tags.

Pyrethroids are also classified as type 2 pyrethroids (e.g., fenvalerate) because they have an alpha-cyano moiety and type 1 pyrethroids (e.g., permethrin), which lack this moiety and thus are less toxic than type 2 pyrethroids. The reader should be aware that these pyrethroid insecticides can exists as chiral and geometrical isomers with some isomers displaying different pharmacokinetic and pharmacodynamic properties from other isomers of the same pyrethroid. Many of these chemicals are stable in weakly acidic water, but very unstable in alkaline medium.

Pharmacokinetics

Dermal absorption of pyrethrins and pyrethroids is indeed very limited (<1%) across the skin of domestic and laboratory animals and humans (Baynes and Riviere, 2001). This is primarily because these chemicals are very lipophilic (Ko/w range from 4.0 to 7.4) and they have large molecular weights. There is therefore the tendency for these chemicals to reside in the outermost layer of the skin (stratum corneum) with little or no penetration into the systemic circulation. For this reason, the pyrethroids are approved by US EPA and *not* the US FDA. These chemicals are also rapidly detoxified to harmless alcohol and acid metabolites and conjugates in the liver and blood with half-lives ranging from a few minutes to typically a few hours (Ray, 2005). The *trans* isomers are hydrolyzed at a faster rate than the *cis* isomers, and the presence of a α-cyano group in the third and fourth-generation pyrethroids also slows down hydrolysis and oxidation. In spite of this, many of these pyrethroids do not result in significant meat or milk withdrawal times following topical administration to food animals (Riviere and Baynes, 1998). For example, there are several approved pour-on products for food animals that contain as much as 1–5% permethrin or 1% cyfluthrin but have zero meat and milk withholding times.

Mechanism of Action

Many different mechanisms of action have been proposed for this class of insecticides. Potential targets in insects include voltage-gated sodium, chloride, or calcium channels, GABA-gated chloride channels, mitochondrial ATPase, and nicotinic receptors, to mention a few of the many proposed targets for pyrethroids. However, the main target is the gating kinetics of Na^+ channels in nerves, which results in repetitive discharges or membrane depolarization and ultimately death of the ectoparasite. Pyrethroids are extremely selective for insects over mammals as insect sodium channels can be as much as

100 times more sensitive than mammalian brain channels (Warmke et al., 1997). In fact, the selectivity ratio (mammalian toxicity vs. insect toxicity) of most pyrethroids exceeds 1000.

Recall that in normal voltage-gated sodium channels it is the inward sodium current that produces the action potential and then it is rapidly closed at normal resting potentials. However, interaction of the pyrethroid with the sodium channel causes the sodium channel to remain open. This leads to a slowing of both the activation and inactivation process, which causes the now modified channel to be in a stable hyperexcitable state. Pyrethroid structure and stereospecificity plays a significant role in the mechanism of action. For example, type 2 pyrethroids such as fenvalerate keep the sodium channel open for a significantly longer period (i.e., prolongation of sodium channel current) than type 1 pyrethroids such as permethrin, which in part explains the differential toxicity between these two classes of pyrethroids. The 1R and 1S *cis* isomers bind competitively while the 1R and 1S *trans* isomers bind noncompetitively to another site. These insecticides, especially the type 2 pyrethroids, could also suppress GABA and glutamate receptor-channel complexes. Pyrethroids are also classified as either type I compounds (permethrin, resmethrin) because they cause rapid onset of hyperactivity and repetitive action potentials or type II compounds (fenvalerate, cypermethrin) because very low doses cause lethal effects and few behavior changes.

Efficacy

Pyrethrins and pyrethroids are formulated in shampoos, sprays, collars, dips, dusts, and ear tags to repel or treat for most of the known ectoparasites important to domestic animals. The discussion below attempts to review the more common application of pyrethroid formulations used in veterinary medicine. Note that these are only examples, and that applications may vary depending on indication (spot-on vs. spray) and this is related to the concentration of the pyrethroid in the formulation. These products may be formulated with synergist and/or insect growth regulators.

First-Generation Pyrethroids

Allethrin (d-trans allethrin) is most often formulated in shampoos for dogs and cats to kill fleas and/or ticks. An excellent example includes Mycodex® SensiCare™ Flea and Tick Shampoo, which has 0.12% d-trans allethrin. First-generation pyrethroids are rapidly metabolized in the body.

Second-Generation Pyrethroids

Phenothrin (85.7%) is frequently used as a spot-on treatment for tick and fleas in dogs only. These spot-on products are *not* approved for cats as of 2005. There are phenothrin flea collars and a shampoo formulation available for both dogs and cats. Resmethrin (0.5%) is more often approved in foggers in stables and kennels, and it also formulated as topical sprays for horses and there are a few shampoo formulations available for control of fleas and ticks on dogs and cats.

Third-Generation Pyrethroids

Permethrin is probably the most widely used pyrethroid in veterinary medicine. Almost all of the spot-on products are at high concentrations and are therefore approve only for dogs, and not cats, to manage fleas, ticks, and mites. The sprays, shampoos, and dusts are formulated at lower concentrations and thus approved for both species. Permethrin is also widely used in food animals as sprays and pour-on to treat for ectoparasites and require short withdrawal times provided the product is correctly diluted and the products are applied no more than every 2 weeks. For example, the Atroban spray products have a 5-day withdrawal time in swine treated with 0.11% permethrin for mange mites but 0 days in cattle treated with 0.02% pemethrin. Many products can have residual activity up to 28 days for some formulations.

Fenvalerate (8%) is formulated in ear tags for cattle to control flies, lice, and ticks. There are no approvals for companion animals.

Fourth-Generation Pyrethroids

Cyfluthrin is approved for use as an ear tag and as a pour-on for cattle (e.g., Cylence®, 1%) with no withdrawal time. As with other pyrethroids there are some concentrates that are approved for use around livestock premises but not directly on animals. These pyrethroids offer the greatest resistance to sunlight exposure. Cypermethrin is usually formulated as a spray and predominantly used as fly repellent around horses with some formulations marketed as being sweat resistant.

Fifth-Generation Pyrethroids

Beta-cyfluthrin is a more potent isomer of cyfluthrin (rat oral LD_{50} = 960 mg/kg). This pyrethroid has been formulated as sprays and ear tags. The ear tags Cylence Ultra™ (8% beta-cyfluthrin) approved for beef and lactating dairy cattle are effective up to 5 months for the control of horn flies, face flies, Gulf Coast ticks, and spinose ear ticks, especially if both ears are used.

Pyrethroid ether

The above pyrethroids are pyrethroid esters, however, in 1987, a new class of related insecticides, known as pyrethroid ethers, were introduced. A good veterinary example of a pyrethroid ether is Etofenprox, which does not contain a cyano group and centers of asymmetry, and therefore has no stereoisomerism as seen in pyrethroid esters. This has been formulated in many dog and cat formulations as sprays and spot-ons with minimal concerns for cats compared to concerns with other pyrethroids in cats. The oral LD_{50} in rats is >42,889 mg/kg and dermal LD_{50} >2140 mg/kg (Harling et al., 1985; Hashimoto et al., 1982). Frontline TritaK for cats (11% etofenprox) is also formulated with fipronil and methoprene in addition to kill fleas, flea eggs, and flea larvae, and starts killing ticks in as little as 1 hour. Of course this chemical combination effectively breaks the flea lifecycle. Etofenprox is also formulated at higher concentrations (e.g., 40%) in other products such Bio Spot Active Care™ Flea and Tick Spot On® for Cats at 40% etofenprox and 3.6% methoprene.

Safety/Toxicity

The selectivity ratio, which compares toxicity in insects with that in mammals, is higher for pyrethrins and pyrethroids (>1000) than for OPs and carbamates (<100). Dermal exposure to pyrethrins and pyrethroids rarely results in systemic absorption to cause significant signs of toxicity in domestic animals, but grooming of the treated hair coat by cats can result in significant oral ingestion of pyrethroids. Cats more so than dogs present with clinical signs of toxicity, and this is oftentimes after the dog formulation was inadvertently applied topically to adult cats or kittens, or the untreated cat comes into physical contact with a treated dog. Recall that cats are deficient in their ability to clear chemicals by hepatic glucuronidation. This explains why cats are more sensitive than dogs to pyrethroids. The dog-only spot-on flea and tick products can contain as much as 45–65% permethrin, which at this concentration can be very harmful to most cats (Meyer, 1999). Not only is permethrin toxicity in cats a great concern, but more recently phenothrin toxicity in cats has also been a concern. Phenothrin, a type 1 pyrethroid similar to permethrin, was originally approved as a spot-on product with 85.7% phenothrin for cats. These products were canceled in 2005 by the manufacturer because of the adverse effects and numerous deaths in feline patients.

Clinical signs of pyrethroid toxicity may be confused with OP or carbamate toxicity, and they include nerve and muscle disorders and can be grouped in type 1 (T) poisoning and type 2 (CS) poisoning. The onset of signs of toxicity can occur within minutes to hours depending on the route of exposure, and the signs may resolve within 24 to 72 hours following supportive therapy. The type 1 syndrome is similar to DDT poisoning in that it involves progressive body tremors, exaggerated startle response, hyperexcitability, and death. Type 2 syndrome is associated with induced salivation, weakness, and distinctive burrowing syndrome. These signs appear in both dogs and cats and large animals, and the prognosis is guarded to good depending on the exposure level. Readers are encouraged to refer to other text and references on appropriate therapy (Richardson, 2000).

Aquatic animals such as fish and invertebrates are highly susceptible to pyrethroids (Smith and Stratton, 1986), therefore their use on and around livestock facilities should ensure there is minimal environmental impact.

Indoxacarb

Inodoxacarb represents the oxadiazine class of insecticides discovered in the 1990s. These pet products, which are used once a month (e.g., Activyl®), are only approved for treating adult fleas, larvae, and eggs but not ticks, lice, mites, and mosquitoes. The products are approved for use in cats and dogs only. The chemical does not need to be absorbed across the skin to get into the systemic circulation for it to be effective. Efficacy is dependent on contact with the flea in the hair coat of the animal.

Mechanism of Action

Once fleas are exposed to indoxacarb, it is rapidly cleaved to its decarbomethoxylated metabolite, which appears to be a potent blocker of voltage-gated sodium ion channels in fleas. There are many other insecticides, such as pyrethroids and organochlorines, that target insect voltage-dependent sodium ion channels, but this chemical has quite distinct actions from the other well-known sodium channel-active commercial insecticides (Lapied et al., 2001). This suggest that there is limited chances for cross-resistance between pyrethroids and indoxacarb products.

Safety/Toxicity

If there is systemic exposure in the pet to indoxacarb, metabolic activation is minor compared to the insect and there are metabolic pathways (indanone and oxadiazine ring cleavage) in mammals that convert indoxacarb mainly to nontoxic metabolites. Furthermore, there is differential sensitivity of sodium ion channels between insects and mammals. Activyl Tick Plus is only for dogs and not cats as this product has permethrin in the formulation, which could cause adverse effects in cats exposed to this product.

Organophosphates

Organophosphates have insecticidal, ascaricidal, and helminthicidal activity, and are thus classified as broad-spectrum ectoparasiticides, which explains why they were used in veterinary medicine for at least four decades since their discovery during WWII. These chemicals are becoming less popular for use in and around domestic animals as there are safer products such as the ivermectins, pyrethroids, neonicotinoids, and firponil. Another concern is related to their persistence in the environment, which can negatively influence the ecosystem. For these and many other reasons, many of the popular OPs which were approved by US FDA or US EPA for use in companion or food animal species are no longer marketed or available for veterinary use. However, there is still sufficient veterinary use of OPs that are US FDA approved (Table 43.3) and US EPA registered and the reader should be aware of their efficacy and be able to recognize signs of OP toxicity in domestic animals.

Chemistry

Organophosphates are usually classified as phosphates (e.g., dichlorvos), phosphorothioates (e.g., coumaphos), or phosphorodithioates (e.g., malathion, fenthion). The term “thioates” indicates that, for some OPs, the double-bonded oxygen is replaced with a sulfur molecule. They can also be classified based on when these chemicals were first developed for commercial use. The aliphatics derivatives were the first OP products and are characterized by their aliphatic like structures (e.g., dichlorvos, malathion). These were followed by the phenyl derivatives, which contain a phenyl ring with one of the ring hydrogens displaced by Cl, NO_2, CH_3, CN, or S (e.g., fenthion, famphur), and then the heterocyclic derivatives where the ring structures are composed of different or unlike atoms such as N or S (e.g., diazonon, chlorpyriphos). The latter group is the longest acting OP based on their chemical structure. The molecular weights of many of these chemicals used in veterinary medicine range from 257 (trichlorfon) to 384 (ethion). These chemicals can be very lipophilic with octanol–water partition coefficients ranging from 2 to 6.

Mechanism of Action

The thioates compounds (P=S) unlike the phosphate (P=O) compounds must first be metabolized in the liver to oxy compounds before they can irreversibly inhibit acetylcholinesterase (AChE) by phosphorylation. Inhibition of AChE results in accumulation of acetylcholine (ACh) at cholinergic receptors. Recall that ACh is a normal neurotransmitter released at synapses that mediates transmission of nerve impulses to effector organs or skeletal tissues to produce a response. AChE is normally present at these nerve endings to rapidly hydrolyze Ach to control the level and duration of synaptic transmission. With OPs inhibiting AChE the resulting accumulation of ACh leads to paralysis and death to the parasite.

Efficacy

As of 2006, there are only 10 OP generics approved for veterinary use in the USA. Systemic bioavailability following topical application is limited, but these chemicals are distributed across the entire coat of the animal by lateral diffusion following topical application to a localized region. Several of these OPs are formulated as topical sprays and dusts or dip application (coumaphos and phosmet), while many are formulated as ear tags in cattle (pirimiphos and diazinon) and flea collars for dogs and cats (tetrachlorvinphos, dichlorvos, and diazinon). Chlorpyriphos and phosmet dips are the only OPs still approved for use in dogs to treat mange. Although chlorpyrifos was recently labeled for restricted use by US EPA, there are several Happy Jack products that are approved for use as shampoos and dips on dogs. These two products are indicated to kill fleas, ticks, and sarcoptic mange mites on dogs. Prior to the availability of avermectins for veterinary use, fenthion (Spotton®) and famphur (Warbex®), which are still US FDA approved drugs, were used extensively to control cattle grubs (*Hypoderma bovis* and *Hypoderma lineatum*) as well as lice. When treating for cattle grubs, the reader should be reminded that application of these therapeutics should occur soon after the heel-fly activity ceases. If the grub larvae are allowed to migrate to the esophagus or spinal canal, rapid killing of large numbers of these larvae following treatment can cause host–parasite reactions such as bloat, salivation, staggering, or paralysis.

Safety/Toxicity

Inhibition of AChE can result in muscarinic and nicotinic effects. The muscarinic effects at the autonomic effector organs include miosis, lacrimation, vomiting, diarrhea, frequent urination, dypsnea, bradycardia, and hypertension. The nicotinic effects include effects at the neuromuscular junction such as muscle tremors and twitching, paresis, and possibly paralysis. Central nervous system signs may include depression, hyperactivity, and seizers, but these are rare events. OP-induced delayed neurotoxicity (clinical signs delayed for 7–21 days) may be observed in dogs and cats following exposure to some but not all OPs. The following drugs may potentiate the toxicity of OPs: phenothiazine tranquilizers, aminoglycoside antibiotics, and neuromuscular blocking agents such as levamisole and nicotine. Brahman, Charolais, and Simmental cattle are more sensitive than English breeds of cattle, greyhounds and whippets are the more sensitive dog breeds, cats are more sensitive than dogs, and poultry are more sensitive than mammals to OPs. Recall that several of these OPs are very lipophilic and potential residues in tissues and milk are possible. Except for fenthion and famphur, many of the currently marketed

OPs approved for use in food animals as of 2006 have a very short or zero withdrawal times as they are less likely to cause residues in meat or milk when used according to label. These are all US EPA registered OPs, and they include coumaphos (Co-Ral®), which has a zero withdrawal time for meat and milk, and phosmet (Del-Phos® Emulsifiable Liquid Insecticide), which has a 3-day and 1-day meat withdrawal time for cattle and swine, respectively. Fenthion and famphur have very long meat withdrawal times which are 46 and 35 days, respectively, and are not approved for us in lactating dairy cattle.

Carbamates

Carbamates are cyclic or aliphatic derivatives of carbamic acid. These insecticides are generally safer than OPs, but they should be used with caution. The mechanism of action of carbamates is similar to that of OPs, except that inhibition is by carbamylation and the reaction is slowly reversible and spontaneous. The most frequently used carbamate is carbaryl, which may be formulated with pyrethrins and/or synergists. These products are only approved for cats and dogs as shampoos (0.5% carbaryl) and dusts (12.5% carbaryl). Unfortunately, these products need to be applied more often than the newer generation of veterinary insecticides and insect resistance is commonly reported. Propoxur is another carbamate that is slightly more potent than carbaryl, but it is included in flea and tick collars for dogs and cats with claims of providing continuous protection against fleas for up to 5–6 months (e.g., Scratchex® for dogs). Toxicity associated with application of these two carbamates can be avoided if pets are not simultaneously exposed to anticholinesterase drugs or pesticides with similar mechanism of action and exposure is limited to cats and dogs older than 12 weeks.

d-Limonene and Linalool

These are volatile oil extracts from the peel of oranges, and it is their vapors, and not necessarily direct contact, that affects ectoparasites. This insecticide is formulated in shampoos and sprays. Although a relatively safe insecticide, **d-limonene** has caused *sporadic toxicoses* in cats (Hooser, 1990; Lee et al., 2002). Clinical signs are not often seen at the label concentration, but can occur at greater than five times the label dose. These signs can include excessive salivation, ataxia, and tremors as well as gross lesions in the scrotal and perineal area.

Amitraz

Amitraz is the only formamidine used in veterinary medicine, and is available in various formulations to treat for ticks, lice, and mites in dogs, swine, and cattle. To date, none of these approved products are labeled to repel or kill fleas. Although the mechanism of action of amitraz is not very clear, this ascaricide inhibits monamine oxidase (MAO) that normally metabolize neurotransmitter amines present in the CNS of ticks and mites. There are no effects on cholinesterase activity.

Efficacy

Amitraz is used as a dip (Mitaban®) to treat for generalized demodicosis (*Demodex canis*) in dogs. Amitraz can be applied as a 0.025% (250 ppm) sponge-on solution once a week (UK) or every two weeks (US). However, this drug is not approved to treat localized demodicosis or scabies. There are several reports describing the use of higher concentrations (e.g., 1.25%) or more frequent applications to treat for scabies (Hugnet et al., 2001). These off-label protocols run the risk of dogs developing severe signs of amitraz-related toxicosis described in Section Safety/Toxicity, and its advisable to use the more safe drugs described for this condition. For example, selamectin is the only approved spot-on drug for treatment and control of scabies in dogs (Shanks et al., 2000). Amitraz at 22.1% is formulated with fipronil (9.8%) and methoprene (8.8%) in a popular product called Certifect® for dogs. It starts killing all stages of ticks within 6 hours of application. It also aids in the control of sarcoptic mange infestations and by applying once per month for at least 2 months it is effective for the elimination of mites. Amitraz is also impregnated in tick collars for dogs (Preventic®, 9% active ingredient), and can kill ticks for up to 3 months with activity beginning within 24 hours of placing the collar on the dog. These amitraz tick collars are not approved for cats. Amitraz (e.g., Taktic® E.C., 12.5% active ingredient) is approved for use in beef and dairy cattle for ticks such as *Amblyomma americanum* and *A. maculatum*, mites such as *Chorioptes bovis, Psoroptes,* and *Sarcoptes scabei,* and biting louse (*Damalinia bovis),* and sucking lice *Haematopinus eurysternus, Lignathus vituli*, and Solenoptes capillatus. This product is also approved for use in swine to treat for mange mites and louse. Taktic® is not approved for use in horses or dogs as it may cause deaths!!! Once the spray mixture has been prepared it must be applied within 6 hours or it will lose its efficacy.

Safety/Toxicity

This drug is not approved for use in cats, chiuahuas, in pregnant or nursing bitches or puppies less than 3 months old (Curtis, 2004). In mammals, Amitraz activates α_2-adrenergic receptors, and adverse effects are similar to those seen with xylazine. Signs of toxicity include CNS depression, bradycardia, polyuria, hyperglycemia, and sedation, which can last for up to 24 hours

(Jones, 1990). Dogs tested with five times or 10 times the labeled dose usually develop most of these signs, therefore the final dip solution needs to be accurately prepared. The adverse effects from amitraz toxicosis can be reversed in dogs with a low dose of atipamezol (50 μg/kg IM), which is an α_2-antagonist (Hugnet et al., 1996). Accidental consumption of amitraz flea collars by dogs has resulted in toxicosis (Grossman, 1993). Swine should not be treated more than four times a year and should not be treated within 3 days of slaughter. Amitraz is a potential hazard to humans (Avsarogullari et al., 2006) if they do not wear appropriate personal protection such as chemical resistant gloves, aprons, and goggles/face shields.

Insect Growth Regulators (IGRs)

These agents first appeared in the market in the 1980s and 1990s, and were popular because the chemicals were promoted as not being a pesticide, and therefore harmless to the pet, livestock, and humans. The oral LD_{50} in rats for these chemicals range from 2 to 10 gram/kg body weight, which supports the extreme safety of these IGRs. These chemical agents affect the developing stages (eggs, pupae, and larvae) of insects and arthropods and do not affect the adult ectoparasite. Because of this, effective control is not achieved until several weeks after treatment and many of these IGRs are often formulated with adulticides described in this chapter. The IGRs can be grouped as juvenile-hormone analogs (JHAs) and insect development inhibitors (IDIs).

Juvenile-Hormone Analogs

The IGRs are juvenile hormone analogs, which falsely signal the organism such as ticks, fleas, and flies to *remain in its immature egg or larval stage* and not develop to adult stage. In the normally developing insect, the hormone levels usually decrease prior to the adult stage. The use of these JHAs therefore falsely signals to the developing insect to remain at its immature stage. Approved JHAs include methoprene, pyriproxifen, fenoxycarb, and cyromazine, with the former two JHAs being the most frequently used in veterinary medicine. Several of these JHAs are available as individual active ingredients in several topical formulations, but they are often formulated with adulticides such as the pyrethrins and/or pyrethroids. *Methoprene* is used mainly to control horn flies in cattle by incorporating it into the cattle concentrate (0.4%) or block (0.01%). This method of application prevents the emergence of adult horn flies from cattle manure. Methoprene is formulated with adulticides for premise flea control, and also formulated with fipronil, phenothrin, or permethrin for flea and mosquito control in dogs and cats. Some of these spot-on applications may contain as much as 2–3% methoprene. *Pyriproxyfen* is often applied as a spot-on, and is effective against flea eggs in cats for up to 56 days (Rust, 2005). This JHA applied every 3 months to cats can not only significantly reduce flea numbers but also 87% of the treated cats did not have fleas after 6 months (Maynard et al., 2001). As with methoprene products, pyriproxyfen is often formulated with adulticides such as permethrin, dinotefuran, and pyrethrins to be applied as sprays, spot-ons, and/or collars for cats and dogs.

Insect Development Inhibitors

The IDIs such as the benzoylphenyl urea compounds (*diflubenzuron, lufenuron*) interfere with the development of the insect exoskeleton by inhibiting chitin synthesis or deposition pathways. Chitin is an essential constituent of flea eggshells and exoskeleton of immature fleas. Following oral ingestion of *diflubenzuron*, it passes unchanged in the feces where it contacts the eggs and larvae of developing flies. *Lufenuron* (*Program*®) is administered orally at monthly intervals to cats and dogs to control fleas. There is also a subcutaneous injectable (10 mg/kg) for cats that is efficacious for up to 6 months against fleas. No adverse effects have been observed in cats, but injectable lufeneron should not be given to dogs as it causes adverse local reactions at the injection sites. As this drug is lipophilic, its slow release from adipose tissues allows maintenance of effective blood levels for several weeks after administration. One of the more popular IDIs is the combination of lufenuron and milbemycin oxime that is used to control fleas on dogs as well as a being a heart worm preventative.

Synergists and Repellents

Synergists such as piperonyl butoxide and *N*-octyl bicycloheptene dicarboximide (MGK 264) are often formulated with topical insecticide products to enhance the activity of insecticides. Almost all of the topicals formulated with these synergist contain pyrethroids and pyrethrins as the primary insecticide. These synergists inhibit oxidative and hydrolytic enzymes in the insects that are responsible for degradation of insecticides to harmless metabolites. It is believed that a carbene derivative forms and binds to the heme moiety of the cytochrome P450 enzyme making it inactive and unable to detoxify the insecticide. This then results in very high levels of the pyrethrin or pyethroid that are toxic and lethal to the ectoparasite (Murray and Reidy, 1989). Synergists by themselves are less toxic than the active ingredients in a topical formulation. Owners and veterinarians should, however, be aware that when cats are exposed dermally to synergists such as piperonyl butoxide at concentrations greater that 1.5%, this may similarly reduce detoxification of pyrethrins in cats and thereby also enhance toxicity of the insecticide in cats (MacDonald and Miller, 1986).

Repellents are also included in formulations to repel insects, although some repellents can be ectoparasiticidal such as permethrin, most repellents are not ectoparasiticidal. The primary advantage of the repellents is that they prevent transmission of vector-borne diseases even if these repellents are not ectoparasiticidal. A repellent can be effective via a number of mechanisms, including inability to enter or land on the hair coat, interfere with or inhibit feeding, and also cause the ectoparasite to become disoriented. Frequently used repellents include butoxypolypropylene glycol (Stabilene), di-n-propyl isocinchomeronate (MGK 326), and diethyl-m-toluamide (DEET).

DEET is an insect repellents approved by US EPA for use in dwellings, on the human body and clothing, on cats, dogs, horses, and pet living/sleeping quarters (US EPA, 1998). There are greater than 225 DEET products registered for use, with DEET concentrations ranging from 4% to 100%. However, DEET is not approved for direct application to the skin/coat of pets, and there are reports of adverse clinical signs in cats exposed to DEET topically (Dorman et al., 1990). Based on data from several experimental dermal absorption studies, DEET may influence the toxicity of insecticides by modulating insecticide dermal absorption (Baynes et al., 1997). Butoxypolypropylene glycol is usually formulated with a pyrethroid insecticide (e.g., resmethrin, permethrin) and/or a synergist (e.g., piperonly butoxide). Most of these formulations are approved for use in horses, cats, and dogs, and formulated as sprays with concentrations ranging from 4.8% to 20%. No adverse effects in pets or laboratory animals have been attributed to this repellent (CA EPA, 2002). MGK 326 is often formulated with pyrethroids and/or synergist to be used as sprays, dips, and shampoos in horses, dogs, and cats. Another safe product, Centaura^{TM} Insect Repellent, which contains 20% picardin, claims to provide 12-hour protection from flies, mosquitoes, and ticks for horses and riders.

Ectoparasiticide Approval and Registration in the US

Unlike other drug classes discussed in this book, either the US EPA or the US FDA Center for Veterinary Medicine (CVM) is involved in the approval of ectoparasiticides. The US FDA CVM evaluates those chemicals that are administered orally or topically but exert their effects following systemic distribution. These chemicals are classified as drugs and many of these drugs require a veterinary prescription. The US EPA evaluates those chemicals applied topically, but exert their effect on the surface of the skin or around the farm premises, and do not often require a veterinary prescription. Table 43.5 summarizes the differences in function between these two critically important federal agencies in the USA.

In spite of these clear distinctions, there is still confusion amongst pet owners and even some veterinarians about how flea products are marketed in the USA as some products can be obtained over-the-counter (OTC) in pet and feed stores while others can only be obtained from the veterinarian. The latter is often referred to as the veterinary-channel products (e.g., Fipronil and Imidacloprid). Even though those flea products may not require a prescription, obtaining these products from a veterinarian only ensures that a client–patient–veterinarian relationship is established, which reduces the probability of

Table 43.5 Comparisons of USFDA CVM and US EPA with regards to ectoparasiticides

	US FDA CVM	US EPA
Mission	Approval and effects of drugs and feed on animals Company files a New Animal Drug Application (NADA) to FDA	Preserve and improve quality of the environment and human health Registration of pesticides Company files an Application for Pesticide Registration (APR) to EPA
Scope	Concerned with drugs that work systemically in the animal This includes any route of administration to the animal	Concerned with anything that affects air, water, and soil quality Chemicals that are applied topically to animals but not absorbed systemically
Safety studies requirements for FDA approval or EPA registration	Drug formulations must be evaluated in same species for which the drug is designated	Active ingredients are tested in lab animals Formulations do not have to be tested in the designated species
Efficacy	Flea control products must be at least 90% efficacious Clinical studies required	Clinical studies not required Practical efficacy in market place is required
Drug distribution	Often by prescription from a licensed veterinarian	Often sold over-the-counter at retail outlets

insecticide toxicoses often associated with OTC products used by pet owners. As discussed in various sections of this chapter, this has often been the problem with OTC pyrethroid and OP products used in cats and dogs.

Another issue that is of concern to the federal government is the extralabel use of ectoparasiticides. The passage of the Animal Medicinal and Drug Use and Clarification Act (AMDUCA) in 1994 allows but does not encourage the extralabel use of approved animal or human *drugs* in special circumstances. As pesticides are not approved drugs, extralabel use of these chemicals is not covered by AMDUCA and extralabel use is strongly prohibited.

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Section 11

Specialty Areas of Pharmacology

44

Chemotherapy of Neoplastic Diseases

Luke A. Wittenburg and Daniel L. Gustafson

The current era of chemical anticancer therapy took hold in the mid-20th century, yet its roots can be traced back 50 centuries to ancient descriptions of neoplastic diseases and the use of medicinal herbs and botanicals in the treatment of these conditions (Papac, 2001; DeVita and Chu, 2008; Morrison, 2010). Nitrogen mustard, the first modern pharmacological chemotherapeutic agent, was developed in the 1940s and additional drugs derived from nitrogen mustard followed shortly after (DeVita and Chu, 2008). The first reported case of treatment of cancer in veterinary medicine occurred in 1946 and involved the use of urethane to treat a dog with lymphoma and lymphocytic leukemia (Innes et al., 1946). The first facility devoted entirely to the treatment of cancer in dogs, the Canine Cancer Clinic, was founded in 1950 and within 10 years had published a review of chemotherapy treatment of spontaneous cancers in canines using numerous agents ranging from nitrogen mustards to cortisone (McCoy, 1958; Morrison, 2010). It was suggested as early as 1962 that the treatment of animal cancer patients had become an obligation of the veterinarian to his or her community (Morrison, 2010). With the advent of animal models of cancer that were predictive for antineoplastic drug activity, drug screening programs were initiated in the 1950s that ultimately resulted in the discovery and development of many of the agents in clinical use today. Since that time, significant advances have been made in the understanding of the biology of cancer and response of specific tumor types to various drugs. While direct translation of discoveries made in human oncology has been hampered, in part by species differences in drug pharmacokinetics, expense, and lack of widespread availability of adequate supportive care in general practices, there has been a significant improvement in the quality, and in many cases quantity, of life for pet cancer patients through improved client education and rational decisions in the selection of appropriate patients while matching therapeutic regimen with therapeutic goal.

Terminology and Treatment Perspectives

Chemotherapy is defined as the application of drugs in an attempt to kill or inhibit the growth of viruses or foreign cells such as bacteria or fungi in the body. Although originating from the individual, cancer cells are viewed as “foreign” in this sense. Recent advances in understanding the biology of cancer and cellular signaling networks important in cancer cell survival and proliferation have led to the development of *targeted therapies*, usually small molecules or antibodies directed at specific cellular receptors or proteins. In contrast to these therapies directed at tumor cells, cancer *immunotherapy* can have an indirect action through potentiation or reactivation of antitumor immune responses that tumors often suppress as a means of escaping immune recognition. Regardless of the type of therapy employed, the treatment regimen chosen must be appropriate for the therapeutic goal for the individual patient. A *cure* is defined as the complete eradication of all tumor cells within the body; in most cases this is not achievable and *palliation,* the reduction of pain and improvement of function to enhance quality of life in the absence of a possibility of cure, has become increasingly recognized as an acceptable therapeutic goal.

The ratio of the toxic dose to the therapeutic dose of a drug is termed the *therapeutic index*. This is often extremely small in cancer chemotherapy as the dose that causes a desired effect, such as reduction in tumor size, is often anticipated to cause some degree of toxicity. Furthermore, the vast majority of chemotherapy drugs are administered at the *maximum tolerated dose* (MTD) which is defined as the highest dose of a drug that can be given without unacceptable or irreversible adverse events and is generally empirically derived in a small population of animals. Toxicities following drug administration in veterinary patients is scored based on guidelines set forth in the Veterinary Co-operative Oncology Group Common Terminology Criteria for Adverse Events

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(VCOG-CTCAE) (VCOG, 2011) and are used to guide dose adjustments for subsequent treatments. More recently, the advent of targeted therapy has given rise to the concept of a *biologically effective dose* (BED), defined as the dose leading to some measurable desired response at the proposed target or a surrogate biomarker. Dosing of chemotherapy drugs in humans and animals is generally based on body surface area (mg/m^2) instead of patient weight (mg/kg). This was originally instituted as a means of normalizing the maximum tolerated dose of drug between species and was based on the idea that physiological processes that influence drug activity in the body correlate better with body surface area than weight. The exception to body surface area based dosing in veterinary medicine is in very small patients where this dosing results in a relatively higher dose and often weight based dosing may be chosen for animals weighing less than 10 kg. The formula for converting weight to body surface area for dogs and cats is:

$$Surface\ Area = \frac{Body\ Weight^{0.67} \times K}{10^4}$$

where surface area is given in square meters and K is a species constant with a value of 10.0 and 10.1 for cats and dogs, respectively. Numerous tables exist for conversion of weight to surface area.

The decision to use a certain chemotherapeutic drug or combination of drugs, with or without additional therapeutic modalities such as surgery and radiation therapy, as well as the assessment of response, is influenced by the therapeutic intent for each individual patient. Therapeutic decisions are influenced by patient factors such as species, breed, age, and health status, neoplasm factors such as histological type, stage and/or grade of tumor, facility factors such as availability of quality follow-up care and available treatment modalities, and pet owner factors such as time commitment, tolerance for adverse effects, and cost of therapy.

Adjuvant therapy, where chemotherapy is utilized following control of the primary tumor by means such as surgery or radiation, aims to limit the spread of micrometastatic disease and is a common application of chemotherapy in veterinary medicine. *Neoadjuvant* or *primary therapy* is the use of chemotherapeutic drugs prior to surgery or radiation as a means of limiting spread of metastatic disease as well as reducing primary tumor burden. In reference to hematopoetic or lymphoid tumors, *induction therapy* is a term referring to the initial utilization of chemotherapy drugs to induce a remission. Following induction of remission, *maintenance therapy* involves the use of chemotherapy with the goal of maintaining a stable remission. When a tumor fails to respond to chemotherapy or a patient comes out of remission, *rescue* or *salvage therapy* is often employed with the goal of achieving a second remission.

Cancer Biology

A basic understanding of some of the aspects of the biological behavior of cancer is important for understanding the therapeutic approaches and the limitations of these approaches due to the striking diversity of neoplastic diseases. The transformation of normal cells to neoplastic cells can be viewed as a multistep process by which normal cells acquire successive capabilities that allow for tumor growth and subsequent spread throughout the body. These capabilities have been described as “hallmarks” of cancer and are traits that differentiate cancer cells from normal cells in the body, the most fundamental being the ability to sustain signaling that allows the cell to continuously proliferate (Hanahan and Weinberg, 2011). In addition to sustaining positive proliferative signaling, cancer cells must also bypass the programs that are designed to inhibit proliferation and maintain homeostasis of cell numbers and normal tissue architecture through control of entry into the cell growth and division cycle (Hanahan and Weinberg, 2011). While the biochemical processes of cell proliferation and passage through the cell cycle are the same between cancer and normal cells, it is the rate and timing of the processes that provide a qualitative difference and results in the utilization of drugs that target rapidly dividing cells as a therapeutic option. Because many of the drugs are most active against cells that are actively dividing, and some chemotherapy agents are more active in specific phases of the cell cycle, an understanding of the activities that occur during each phase of the cell cycle is important. The cell cycle is generally divided into four phases: G_1, S, G_2, and M, with cells that are at rest or not actively dividing termed G_0. The G_1 phase is a period of cell growth, active gene transcription, and protein synthesis and is required for building up the “biomass” required for cell division. This phase is followed by the S phase, a period of DNA replication and chromosome duplication in preparation for mitosis. During the G_2 phase the cell continues to grow in size and in M phase the cells undergo active mitosis. Prior to the start of the S and M phases of the cycle are two important checkpoint mechanisms, which cells utilize to ensure that the cell is prepared to carry out chromosome duplication and mitosis, and these checkpoints are often bypassed in cancer cells allowing for uncontrolled growth. Figure 44.1 depicts the cell cycle specificity of the major classes of drugs that are used in veterinary medicine. Other hallmarks of cancer have also provided some potential therapeutic targets. While sustaining positive proliferative signaling and evading growth-suppressive signaling are important for cancer on the cellular level, in order to grow into a clinically relevant mass a tumor must induce angiogenesis, or the formation of new vasculature, in order to provide for the increasing demand for nutritional support (Folkman,

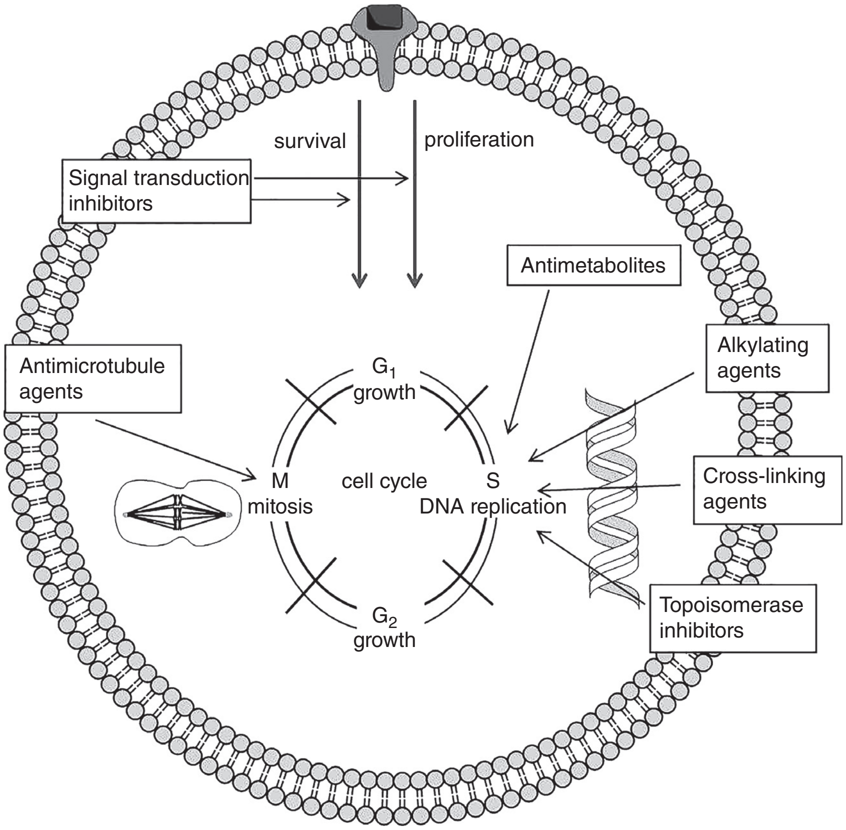


Figure 44.1 Cellular targets of the common classes of chemotherapeutic agents used in veterinary oncology and their sites of activity.

2002). Targeting tumor vasculature has been proposed as a way to control tumors by effectively starving them to prevent further growth (Kerbel and Folkman, 2002). The term *metronomic chemotherapy* refers to the use of low-dose, continuous administration of conventional cytotoxic drugs without breaks in treatment and has been utilized successfully in human and veterinary oncology as a means to delay tumor recurrence or slow growth (Elmslie et al., 2008; Burton et al., 2011; Romiti et al., 2013; Schrempp et al., 2013).

While uncontrolled cellular growth and passage through the cell cycle are a hallmark of cancer cells, the growth rate of a tumor in the clinical setting does not necessarily directly correlate with the rate at which cells are traversing the cell cycle. Early studies into the kinetics of cell proliferation in experimental tumors demonstrated that tumor growth rate is rapid initially but then slows as the fraction of cells entering the cell cycle reduces and cell death increases, potentially due to reduced availability of nutrient supply in bulky tumors; in each examined stage of tumor growth the length of the cell cycle itself remained unchanged (Frindel et al., 1967). Clinically, many tumors in veterinary medicine are diagnosed when they are relatively advanced and thus there may be only a small fraction of cells that are actively undergoing cell division, which has implications for effectiveness of therapy when utilizing drugs that have their maximal effect on actively dividing cells. In the 1960s, studies in mice utilizing leukemia cell lines demonstrated that a single cell was capable of generating a tumor and that a cure could only be attained by eradication of all tumor cells (Skipper et al., 1964). Furthermore, it was determined that any effective dose of chemotherapy killed cells by log kill kinetics, which led to the *fractional kill hypothesis*. This states that with each dose of chemotherapy a constant fraction of cells is killed, as opposed to a constant number of cells, regardless of the size of the original tumor and that successful therapy is then dependent upon the number of cells present at the beginning of treatment. Thus, in theory, with the application of multiple doses of chemotherapy it should be possible to attain a cure. In practice this is not always the case as the fraction of cells killed with many of the available drugs is small, resistance to therapy often occurs, and toxicities prevent rapid successive administration of drugs. However, these concepts illustrate the importance of early detection in a successful treatment outcome as smaller tumors are likely to have a larger dividing fraction and the fractional kill hypothesis implies that a cure could be achieved earlier in the course of treatment for smaller tumors with fewer cells present.

Chemotherapy of the Cancer Cell

Tumor Cell Sensitivity

Increased understanding of cancer cell biology has led to the evaluation of a number of novel targets for therapy. The targets for traditional cytotoxic chemotherapy include DNA (through targeting of nucleotide bases, enzymes involved in DNA synthesis, and DNA damage repair), microtubules, and growth factor receptors. More recently discovered targets include products of overexpressed oncogenes, antiapoptotic proteins, cell-cycle regulators, proteasomal machinery, cell surface antigens, and enzymes involved in epigenetic modulation of DNA. While cancer in veterinary patients may share many of the same putative targets as human cancers, translation of newer "targeted" therapies into veterinary medicine has lagged behind development in human oncology, with a couple of notable exceptions. This chapter will focus on the drugs that are currently in primary use for veterinary oncology.

Individual cell sensitivity to a drug is dependent upon a number of factors, such as absorption, distribution, and metabolism, entry into the cell or affinity of interaction with a cell-surface target, the type of lethal or sublethal damage induced, and the cellular response to this damage. Altered tumor vasculature and differences in intratumoral pressure or drug binding can affect drug uptake in a negative or positive way. Some drugs can enter the cell through passive diffusion due to the lipophilic nature while others may utilize active transport via an array of carriers such as amino acid transporters (melphalan), nucleoside transporters (gemcitabine), or reduced folate carriers (methotrexate)(Goldman et al., 1968; Mackey et al., 1998; Yanagida et al., 2001). The importance of active transport mechanisms for entry into the cell is that reduced entry, and thus diminished efficacy, can occur with administration of other substrates or analogs that block drug transport. Many cytotoxic drugs exhibit greater toxicity in rapidly dividing cells and are termed *cycle specific.* There are a few drugs that have similar effects on proliferating and nonproliferating cells, including the nitrosureas (cyclohexylchloroethylnitrosourea, CCNU, and bischloroethylnitrosourea, BCNU)(Twentyman and Bleehen, 1975). Because they do not require cells to be proliferating to exert lethal effects these drugs are termed *cycle nonspecific.* Other cytotoxic drugs may demonstrate variations in lethal toxicity depending on the phase of the cell cycle and are termed *phase specific.* Vinca alkaloids and taxanes are examples of drugs that exert lethal damage exclusively in cells undergoing mitosis as they target the dynamics of microtubule formation. For drugs that have a very direct and specific interaction with a target molecule, the cellular levels of the target can affect the intrinsic sensitivity of the cell. For example, doxorubicin and other anthracyclines interact with topoisomeraseII-α (TopoII) and reduced levels of this target have been identified in populations of doxorubicin-resistant cells (Withoff et al., 1996). Furthermore, amplification of the gene encoding TopoII, leading to increased target expression, has been identified as a potential marker of enhanced sensitivity and improved outcome with anthracycline-based chemotherapy in human breast cancer patients (Park et al., 2003). For drugs that directly target DNA, the sensitivity of a cancer cell is dependent upon the balance between formation of DNA lesions and repair of the lesions. Alkylating agents such as cyclophosphamide exert their toxic effect by inducing DNA strand breaks following the formation of O'6-methylguanine; the enzyme O'6-methylguanine-DNA methyltransferase (MGMT) is a conserved protein that is capable of directly reversing this lesion and levels of MGMT have been proposed as a predictor of tumor sensitivity to alkylating agents in humans (Hansen et al., 2007; Xu et al., 2014). Often, the final effector of cancer cell death following exposure to cytotoxic drugs is the activation of apoptosis, or programmed cell death. The activation of apoptosis in a cell following a toxic insult is dependent upon the balance of pro- versus antiapoptotic signaling, and alterations that shift the balance in the direction of antiapoptosis, as has been demonstrated in human and canine lymphoma with elevated levels of the antiapoptotic protein survivin, have a negative impact on chemosensitivity (Ambrosini et al., 1997; Rebhun et al., 2008). Thus, the proximity of a cancer cell to the "apoptotic threshold", determined by the interplay between the pro- and antiapoptotic signals, can be a key determinant of therapeutic responsiveness.

Currently, there are efforts to utilize the in vitro chemotherapeutic sensitivity of large panels of cell lines, such as the NCI 60, along with gene expression data from these cells, to begin to build models that might predict sensitivity of other cell and tumor types based on similarities in gene expression signatures, and validation of these models may allow the future administration of cytotoxic agents in a more personalized manner (Scherf et al., 2000; Lee et al., 2007).

Tumor Cell Resistance

Resistance to chemotherapy is the major mechanism of therapeutic failure in cancer drug therapy. Many of the hallmarks of cancer and the genetic instability of cancer cells provide mechanisms whereby cells can tolerate and sustain the stresses induced by cytotoxic drugs. Resistance can be either intrinsic to the cell or acquired through the selection and subsequent expansion of resistant clones as sensitive cells are killed. Intrinsic resistance mechanisms are those that are constitutively expressed in the tumor tissue from the outset of therapy as they are generally derived from the normal tissue from which the tumor developed. As a component of their barrier function, epithelial tissues generally express numerous transport and detoxifying enzymes for xenobiotics, and carcinomas derived from these tissues also often express increased levels of these enzymes leading to a more resistant phenotype. Conversely, tumors derived from normal tissues with lower levels of defense mechanisms, such as hematopoietic or lymphoid tissues, are relatively more chemosensitive than epithelial tumors. As mentioned in the previous section, altered drug uptake can influence the sensitivity or resistance level of a cancer cell to drugs such as melphalan, methotrexate, or nucleoside analogs such as gemcitabine. Some cytotoxic agents are administered as prodrugs and require metabolic or catabolic activation to an active form to result in cell death. Reduced activation has been identified as a potential mechanism of acquired resistance for the nucleoside analog group of drugs and has been suggested as a mechanism for resistance to the alkylating agents cyclophosphamide and ifosfamide (Spears, 1995; Zhang et al., 2005). When administered as single agents, that is not as part of a combination therapy protocol, resistance to some drugs manifests as a direct modification of the drug target. Resistance to antimetabolite drugs such as methotrexate and 5-fluorouracil has been found to involve either direct mutation of the target enzyme that reduces binding affinity or mutation that results in decreased levels of the target directly (Gonen and Assaraf, 2012). Similarly, target-based mechanisms of resistance have been identified for the microtubule binding drugs such as taxanes, vincristine, and vinblastine. As these drugs disrupt normal tubulin dynamics through their interaction with β-tubulin, increased expression of different cellular isoforms of β-tubulin with lower binding affinity has been identified, as has mutation in the target β-tubulin that also results in reduced binding affinity (Monzo et al., 1999; Rebucci and Michiels, 2013). For the group of drugs that target DNA replication through interactions with topoisomerase I or II, mutations in the gene encoding the topoisomerase enzyme have been identified, and cells with reduced levels of topoisomerase II demonstrate resistance to doxorubicin (Pilati et al., 2012; Tomicic and Kaina, 2013).

In addition to qualitative and quantitative alterations in the interaction of cytotoxic drugs with their targets, resistance can also develop through increased metabolism or detoxification of a drug by cancer cells. Many of the currently used cytotoxic agents interact with DNA through electrophilic interactions, making them sensitive to thiol-mediated inactivation by conjugation with nucleophiles such as glutathione (Tew, 1994). Induction of the enzyme responsible for conjugation of glutathione to cytotoxic drugs, glutathione S-transferase (GST), has been demonstrated in leukemia cells that have acquired resistance to alkylating agents and there is evidence that this may also be a mechanism through which tumors can acquire resistance to platinum agents such as cisplatin (Schisselbauer et al., 1990; Tew, 1994). In addition to detoxification through conjugation, metabolic inactivation of drugs is implicated as a mechanism of resistance in some instances. Acquired resistance to the nucleoside analog 5-fluorouracil can be associated with elevated cellular levels of dihydropyrimidine dehydrogenase (DPD), the main enzyme responsible for metabolism of fluorouracil to an inactive compound, and DPD activity in tumor tissue has been utilized as a potential marker in determining whether a particular tumor is likely to demonstrate resistance to therapy (Baba et al., 2003; Rebucci and Michiels, 2013).

As the cellular target of many cytotoxic agents is ultimately the DNA, alterations in the response to DNA damage will determine the fate of the cell exposed to these drugs. In normal cells, the DNA damage response is intimately related to cell cycle progression, and detection of damaged DNA generally leads to cell cycle arrest to allow for repair or, if damage cannot be repaired, apoptosis. Resistance to cytotoxic agents can occur through up-regulation of DNA repair processes that abrogate the effects of DNA damaging agents or through down-regulation of damage recognition, both of which act to prevent induction of apoptosis. Altered activity of repair processes specific to double-strand breaks, single-strand breaks, single-nucleotide excision, and base mismatch repair have all been found to play some role in the acquired resistance to a diverse group of cytotoxic agents, including the anthracyclines, camptothecins, alkylators, and platinum agents (Parker et al., 1991; Fink et al., 1998; Pommier et al., 1999; Martin et al., 2008; Helleday, 2010). Some general mechanisms of acquired resistance to cytotoxic agents are listed in Table 44.1.

Table 44.1 General mechanisms of acquired resistance leading to therapeutic failure for some cytotoxic agents

Mechanism	Drugs/classes involved
Reduced uptake	Methotrexate, gemcitabine, cisplatin, melphalan
Increased efflux	Doxorubicin, vinca alkaloids, taxanes
Decreased activation	Antimetabolites (nucleoside analogs)
Increased or decreased levels of drug target	Doxorubicin, methotrexate
Target mutations/reduced binding	Vinca alkaloids, methotrexate
Drug inactivation/ detoxification	Alkylating agents, cisplatin
Increased damage repair	Alkylating agents, cisplatin, doxorubicin
Intracellular redistribution of drug	Doxorubicin

Table 44.2 Examples of ABC transporters implicated in MDR and cytotoxic agents identified as substrates

Transporter	Substrates
MDR1	Doxorubicin, vinca alkaloids (vincristine, vinblastine), taxanes, methotrexate, mitomycin C, epipodophyllotoxins
MRP1	Doxorubicin, vinca alkaloids, paclitaxel, mitoxantrone
MRP2	Cisplatin, vinca alkaloids, doxorubicin, mitoxantrone, methotrexate
BCRP	Mitoxantrone, camptothecin, methotrexate, etoposide, doxorubicin[a], methotrexate[a]

[a] Identified as substrates for mutated forms of the protein but not wild-type (Meyer et al., 2011).

ABC, ATP-binding cassette; BCRP, breast cancer resistance protein; MDR, multiple drug resistance; MRP, multidrug resistance-associated protein.

One very concerning mechanism of acquired resistance results in a phenotype in which cancer cells become resistant to a variety of structurally and mechanistically diverse drugs. While alterations in DNA repair processes or detoxification via glutathione conjugation can certainly lead to resistance to more than one drug, the classic mechanism resulting in the multiple drug resistance (MDR) phenotype involves the increased removal of drugs from the cell. This is more commonly encountered following therapy with multiple drug protocols where resistance does not appear to occur simply through a combination of target-directed alterations that confer resistance to the individual components of the protocol, but through a more global phenotypic change toward chemoresistance (Pritchard et al., 2012). The MDR phenotype generally refers to the increased expression of energy-dependent integral membrane transport proteins that function to remove drugs from the cell following exposure to chemotherapy (Table 44.2). The major family of proteins that has been implicated is the ATP-binding cassette transporter (ABC) family, comprising approximately 48 proteins, of which 15 are known to have chemotherapeutic drugs as substrates (Fletcher et al., 2010). The most well studied of these proteins is P-glycoprotein, a product of the MDR1 gene (*ABCB1*), which has been implicated in resistance to a number of lipophilic cytotoxic drugs including the vinca alkaloids, anthracyclines, epipodophyllotoxins, taxanes, actinomycin D, topotecan, methotrexate, and mitomycin C (Fletcher et al., 2010). Aside from being expressed in some tumor tissues, *ABCB1* is also expressed in normal cells that line tubules and ducts, serving a role that allows for removal or prevention of uptake of xenobiotics into these cells. Tissues such as kidneys and adrenals, liver and pancreas, endothelial cells (particularly those of the blood–brain barrier), small intestine and colon, and testes all express varying levels of *ABCB1*. Therapeutic implications of the expression of *ABCB1* in nontumor tissues include reduced absorption of orally administered substrates requiring intravenous administration to achieve effective levels and hastening the elimination of substrates from the kidney and liver. Importantly, a loss of this normal barrier function has been demonstrated to result in increased toxicity from substrate drugs such as that seen in Collie dogs and related breeds following exposure to ivermectin or loperamide manifesting as neurotoxicity (Mealey et al., 2001; Sartor et al., 2004). In addition, increased toxicity of substrate chemotherapeutic drugs has been identified in the same population even at reduced doses (Mealey et al., 2003), and pharmacokinetic modeling suggests that this population of dogs may not be able to safely receive substrate drugs such as doxorubicin at doses that are tolerable yet retain efficacy (Gustafson and Thamm, 2010). A more complete review of the therapeutic implications of the MDR1 gene has been published previously (Mealey, 2004) and is discussed in Chapter 50.

The other ABC transporters that have been studied the most extensively with regard to a role in acquired resistance to chemotherapy are the BCRP (*ABCG2*), MRP1 (*ABCC1*), and MRP2 (*ABCC2*) proteins (Wu et al., 2011). The importance of these transporters has been less extensively studied and their importance in the MDR phenotype in veterinary cancer therapy is less well defined. Another transporter that does not belong to the classical ABC family of membrane proteins has been implicated in resistance through redistribution of drug within the cell resulting in accumulation away from the target. The major vault protein (MVP/LRP) has been found to cause sequestration of anthracycline drugs within cytoplasmic organelles preventing their interaction with topoisomerase enzymes (Kickhoefer et al., 1998). Clinical studies from human oncology have attempted to address MDR in cancer through administration of various inhibitors of ABC transporters, most often ABCB1; however, results have been disappointing and explanations for these results include the functional redundancy of some transporters in addition to the fact that often more than one is up-regulated, as well as an increase in systemic toxicity most likely due to altered pharmacokinetics with regard to reduced clearance mechanisms (Fletcher et al., 2010).

Combination Chemotherapy

Veterinary cancer patients may frequently be treated with a combination of chemotherapy drugs or with multimodal therapy, which combines chemotherapy with other modalities such as surgery or radiation therapy. Combination therapy mainly provides a mechanism whereby tumors are exposed to increased dosages of drug which subsequently provides for increased fractional cell kill. The term *summation dose intensity* (SDI) is used to express the relationship between the number of agents in a combination and the overall dose, and can be increased through either adding more agents to the combination or increasing the dose of individual agents. For a combination chemotherapy protocol to provide a therapeutic advantage, the increased toxicity to cancer cells must be greater than any increase in damage to critical normal tissues. The implementation of combination therapy in clinical practice is based in the early studies of leukemia models in mice, which were then translated into treatment of childhood leukemia and resulted in the first major improvements in both the rate and duration of remission and resulted in the first responses that were compatible with a cure (Law, 1952). One of the driving factors in development of combination therapies relates to heterogeneity within tumors with regard to cytokinetics; with a tumor mass containing both cycling and noncycling cells, the use of cycle-specific agents to kill mitotically active tumor cells and cycle nonspecific agents to kill noncycling tumor cells was empirically employed. Furthermore, the reduction of tumor mass using cycle nonspecific drugs may actually increase the growth fraction of a tumor through various mechanisms and increase the sensitivity to cycle-specific drugs (Frei and Antman, 2000). The rational development of combination protocols is based on a number of factors, such as independent or nonoverlapping toxicities, allowing for use of each drug at a full dosage, and each component of the combination should demonstrate some degree of efficacy when used as a single agent. In addition, use of drugs with nonoverlapping mechanisms of action and resistance may provide an improved therapeutic benefit when used in combination. Consideration should be given to the schedule of drug administration (i.e., the order in which drugs are given and timing between drugs) in combination therapy as the effects of the schedule can be quite substantial, although it is less well understood than the effects of dose. The best studied example of the superiority of combination therapy in veterinary oncology is in the treatment of lymphoma in dogs, in which combination protocols provide an improved response rate and duration of first remission when compared to numerous single-agent protocols (Chun, 2009).

Pharmacodynamic Response

With regard to cytotoxic chemotherapy protocols, pharmacodynamic considerations relate to standard measures of response and in veterinary medicine have been adopted from the Response Evaluation Criteria in Solid Tumors (RECIST) guidelines used in human oncology (Eisenhauer et al., 2009). Terms that are important in the clinical evaluation of cancer therapeutics involve measurements of tumor shrinkage and tumor burden versus disease progression:

Complete response (CR): complete disappearance of all target lesions and resolution of symptoms of disease.

Partial response (PR): decrease in the volume of target lesions by 50% or decrease in the maximum diameter of >30%.

Stable disease (SD): neither an increase nor decrease in tumor size or disease symptoms (>20% change in tumor diameter).

Progressive disease (PD): increase in tumor volume of >25% or increase in maximum diameter of >20% *or* appearance of new lesions.

Median duration of response/survival (MDR/MDS): median value for a group of animals treated with a given therapy in terms of the length of time they achieved a complete or partial response or length of survival following initiation of therapy.

Progression-free interval/progression-free survival (PFI/PFS): the amount of time elapsed without evidence of either progressive tumor growth (PFI) or survival without progressive growth of tumor measured from the time of initiation of therapy (PFS).

Disease-free interval/disease-free survival (DFI/DFS): the amount of time elapsed without any disease recurrence (DFI) or the duration of survival of the patient following initiation of therapy (DFS).

With the introduction of newer targeted agents in veterinary medicine, pharmacodynamic responses can also be evaluated by detection of direct inhibition of the molecular target. Studies of the c-KIT inhibitor toceranib have demonstrated that, when used at clinically effective doses, reductions in the amount of activated KIT protein can be detected and these changes correlate with the plasma concentrations of drug (Pryer et al., 2003). Identification of these types of biomarkers of drug activity and their correlation with plasma concentrations may become more important as the optimal dosing of these types of targeted therapies may not be based on MTD but on the biologically effective dose required to inhibit a molecular target.

Drug Toxicity

Aside from the determinants of sensitivity and mechanisms of resistance described above, failure of a given therapeutic protocol may also be a result of treatment discontinuation due to unacceptable toxicity. Phase I studies specific to companion animals for a number of cytotoxic agents have been performed and the MTD defined from these studies is generally the suggested starting dose. However, there is substantial inter- and intraindividual variability with regard to efficacy and toxicity following equivalent doses in patients. For example, in human patients following standard doses of some drugs the variability in drug exposure can range between two and tenfold (Masson and Zamboni, 1997). The pharmacokinetic parameters of many anticancer agents are associated with outcome in terms of response and toxicity, and the high degree of variability makes for inaccurate predictions of response based solely on dose administered. A complete review of the reasons for pharmacokinetic variability in cancer is beyond the scope of this chapter, but patient variability with regard to PK and patient physiology and altered physiological states has been reviewed elsewhere (Undevia et al., 2005; Martinez and Modric, 2010; Modric and Martinez, 2011).

With a few drug-specific exceptions, cytotoxic chemotherapy drugs generally produce a characteristic set of toxicities to various tissues that are often related to the tissue growth rate; given appropriate time most if not all of these tissues will recover. In fact, current convention of scheduling in combination protocols is determined by the length of time it takes for normal tissues to recover following cytotoxic insult. In addition, it is the development of improved supportive care procedures that often allows for more dose-intense treatment protocols. Thus, the selective therapeutic advantage of "nontargeted" or general cytotoxic agents lies in the fact there is often more rapid and complete repair of damage in normal cells than cancer cells.

Toxicities that develop following chemotherapy administration can be placed into three categories: (i) acute toxicity that generally occurs during treatment or very shortly thereafter, (ii) acute-delayed toxicity which generally occurs within 2–14 days following administration, and (iii) chronic or cumulative toxicity that can develop anywhere from weeks to years following administration. Acute toxicity can manifest as hypersensitivity reactions that occur during infusion and result from histamine release caused by either the active drug (L-asparaginase, doxorubicin) or from vehicle-mediated mast cell degranulation (paclitaxel and etoposide). Treatment of this hypersensitivity often involves stopping administration of the drug and general symptomatic therapy for allergic reactions and anaphylaxis such as antihistamines and/or steroids. Routine pretreatment with antihistamines in patients with known hypersensitivity to a given drug may also be effective in preventing acute reactions. Acute reactions may also present as nausea and vomiting that can be intrinsic to the drug (cisplatin) or a function of a rapid rate of infusion (doxorubicin). Pretreatment with antiemetic drugs and increasing length of infusion can be effective means to prevent acute vomiting. Examples of potential cumulative/chronic toxicities include dilated cardiomyopathy in dogs when cumulative doxorubicin doses exceed 180 to 240 mg/m^2, hepatic dysfunction following administration of CCNU, and renal failure following cisplatin use in dogs or doxorubicin in cats. It is important to understand that these toxicities could potentially develop after only a single administration and thus do not always present following chronic administration or follow a protracted course of appearance.

Careful evaluation of clinical and laboratory indices prior to administration and careful monitoring during treatment, as well as prudent choice of drug, are critical in preventing or reducing severity of these toxicities. Secondary malignancies are reported for some of the cytotoxic agents due to their mitogenic potential following DNA damage. This is not commonly encountered in veterinary medicine as the length of time between drug administration and the development of a secondary malignancy is often longer than most pets survive. Here again there are differences in the ability to cause secondary malignancies between drugs with the alkylating agents being the most mutagenic and vinca alkaloids among the least mutagenic. The delayed acute effects are most commonly encountered following cytotoxic chemotherapy and some degree of these effects is generally expected. Tissues that seem to be particularly affected following the majority of cytotoxic agents include bone marrow, gastrointestinal mucosa, gonads, and hair follicles (tissues in which cells require constant renewal due to a short lifespan). Lymphocytes and bone marrow cells appear particularly sensitive to cytotoxic agents and neutropenia is often the dose-limiting toxicity (DLT). The low point in neutrophil count following chemotherapy is referred to as the *nadir* and is generally thought to occur 7–14 days following treatment with most drugs. Severe neutropenia can adversely affect treatment as it can result in dose reductions, treatment delays, and sepsis. Careful monitoring of leukocyte count is critical for appropriate management of neutropenia with regard to appropriate supportive care and future dose determination. Consideration of drugs used in combination protocols must account for the propensity of specific drugs to result in neutropenia as some drugs are more prone to result in significant myelosuppression (cyclophosphamide, carboplatin, doxorubicin, CCNU, vinblastine) while others may cause minimal neutropenia (bleomycin, vincristine in dogs, L-asparaginase). In cases of severe myelosuppression the administration of a recombinant granulocyte-colony stimulating factor (G-CSF) can increase circulating neutrophils by stimulating progenitor cell proliferation and differentiation (Fernandez-Varon and Villamayor, 2007).

In addition to the anticipated myelosuppression following cytotoxic chemotherapy, gastrointestinal toxicity is another common sequela and presents as nausea/vomiting and diarrhea. Gastrointestinal toxicity in dogs and cats is often not as predictable as it is in humans given cytotoxic chemotherapy, and in many cases this is self-limiting and can be managed with general supportive care. In particularly sensitive dogs and cats GI toxicity may be severe and may require intervention such as antiemetics and/or antimicrobials or result in dose reduction. Metronidazole and tylosin are common choices of drugs for chemotherapy-induced diarrhea; however, if gastrointestinal symptoms accompany a febrile neutropenia then antibiotic choice will need to consider bacteria likely to result in sepsis.

Unique toxicities specific to some drugs are discussed in the sections on each drug. Several of the commonly used cytotoxic agents can cause phlebitis and have the potential to create necrosis and tissue sloughing if extravasation occurs. Thus, it is critical that venipuncture and catheter placement are done appropriately and sedation or treatment delay may be necessary if this cannot be performed. Extravasation injury may occur up to 2–4 weeks following treatment and can continue for up to 4 months. Treatment will depend on the agent used; doxorubicin which causes the most severe extravasation injury should be treated by aspiration of any fluid present through the catheter or needle followed by ice-pack treatment three to four times per day. Extravasation of vinca alkaloids should also be addressed by aspiration of any liquid still present in the local area followed by heat therapy three to four times per day. In cases of doxorubicin and vinca alkaloid extravasation, intravenous dexrazoxane or local hyaluronidase administration, respectively, may reduce the subcutaneous toxicity (Schulmeister, 2008). Specific treatment guidelines for common chemotherapy induced toxicities encountered in veterinary oncology have been reviewed in more detail by others (Vail, 2009; Gustafson and Page, 2013).

Hair loss is not a common side effect across all breeds of dogs or cats following chemotherapy but alopecia may be anticipated in breeds of dogs with continuously growing haircoats such as poodles and mixed-breed dogs of poodle lineage, as well as some terriers and Old English Sheepdogs. Cats may lose whiskers following chemotherapy. Regrowth may begin within 1 to 2 months following therapy; however, pet owners should be informed that hair regrowth may occur with slightly different color or texture than the original hair. Communication with pet owners regarding potential side effects prior to selection of a chemotherapeutic protocol is of utmost importance in reducing the distress that is experienced when a pet develops toxicity.

In addition to toxicity experienced by the pet, exposure of the therapist should be considered when administering chemotherapy. Special consideration must be given for proper storage, preparation, administration, and disposal of cytotoxic drugs. The preparation of these drugs should be performed under strict guidelines that involve the use of appropriate personal protective equipment including gloves, masks, and chemical fume hoods. Specific guidelines for cytotoxic drugs are available from the Occupational Safety and Health Administration (OSHA). The potential for exposure of pet owners to chemotherapy residues following treatment generally involve exposure to urine or feces and variable levels of drug may be present in urine depending upon the particular drug and time since administration (Knobloch et al., 2010). Owners should be informed of the potential risk and importance of careful avoidance, collection, and disposal of urine and feces. Young children, pregnant women, and immunocompromised individuals should be instructed to avoid pet wastes for a defined period of time following chemotherapy administration.

Specific Chemotherapeutic Drugs

Alkylating Agents

The alkylating agents were the earliest nonhormonal drugs utilized in the treatment of cancer and comprise a chemically diverse group of compounds. The mechanism of action of the alkylating agents is to interfere with the accessibility of DNA base pairs during replication through the covalent bonding of alkyl groups (e.g., $-CH_2Cl$) to electron-rich nucleophilic groups such as phosphate or hydroxyl moieties. Although a large number of cellular macromolecules contain nucleophilic groups, which are potential sites of alkylation, it is the alkylation of DNA bases that is the major cause of cell death following treatment with alkylating agents. The N-7 and O'6 position of guanine are often a site of alkylation and the net effect can be the loss of guanine from the DNA strand with a resultant strand break. Because their action in causing DNA strand breaks resembles that of radiation, the alkylating agents are frequently referred to as radiomimetics. These drugs can contain one or two reactive groups and are classified as mono- and bifunctional, respectively. The bifunctional alkylating agents are capable of producing inter- or intrastrand DNA cross-links as their major mechanism of cytotoxicity. As a group, these drugs have limited cell cycle specificity and sensitivity of cells is relative to the area under the concentration time curve. In general, the alkylating agents are most useful in tumors of lymphoreticular origin and are less commonly used in sarcomas or carcinomas. Resistance commonly develops after prolonged use and can be drug-specific or across the alkylating class as a whole. Detoxification of these drugs by conjugation with nucleophilic glutathione prevents these drugs from interacting with DNA and increased levels of glutathione or elevated GST can be expected to result in resistance to all drugs within this class. Similarly, increased repair of DNA damage, as is seen with elevated levels of MGMT, can reduce sensitivity of cancer cells to all alkylating agents. Alternatively, resistance that is produced through reduced drug penetration into the cell can be drug specific; for example,

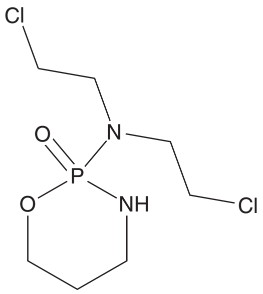


Figure 44.2 Chemical structure of cyclophosphamide, a nitrogen mustard alkylating agent.

melphalan is taken into the cell by the leucine transport system (Del Amo et al., 2008) whereas mechlorethamine is taken in by the choline transporter (Fry and Jackson, 1986) and altered transport affecting one of these drugs does not necessarily cause a reduced efficacy of the other.

Nitrogen Mustards

Cyclophosphamide

The most widely used nitrogen mustard in veterinary medicine is cyclophosphamide (Cytoxan, CTX) (Figure 44.2). Cyclophosphamide is a prodrug that must be converted by the hepatic mixed function oxidase system (cytochrome P450) to an intermediate compound, 4-hydroxycyclophosphamide, which then undergoes spontaneous degradation to the active compound phosphoramide mustard (Cohen and Jao, 1970). Because of the requirement of cytochrome P450 enzymes in the activation of cyclophosphamide, consideration must be given to potential drug–drug interactions in animals coadminstered either inhibitors or activators of these enzymes. The major dose-limiting toxicities in dogs and cats are myelosuppression and neutropenia with thrombocytopenia to a lesser degree. GI toxicity can also be of concern but is more commonly encountered in cats (Fetting et al., 1982). Cyclophosphamide, along with the other nitrogen mustards, is capable of producing alopecia in susceptible animals and causing loss of whiskers in cats. Sterile hemorrhagic cystitis is a potential toxicity that is encountered relatively infrequently and is more likely to develop in dogs than in cats. A metabolite of cyclophosphamide, acrolein, is thought to be responsible for irritation to the bladder following CTX administration. Intravenous administration of furosemide concurrently with cyclophosphamide has been shown to reduce the incidence of sterile hemorrhagic cystitis in dogs (Charney et al., 2003), and when given orally by owners it should be administered in the morning, water intake should be high, and the animal should be given ample opportunity to urinate throughout the day. Administration of MESNA (2-mercaptoethanesulfonate) has also been used prophylactically to bind and detoxify the urotoxic metabolites of cyclophosphamide and ifosfamide (West, 1997). If cystitis occurs with administration of cyclophosphamide the drug should be discontinued and another alkylating agent used in its place (such as chlorambucil). Although maximum plasma concentrations and overall exposure (AUC) to the parent molecule (CTX) are significantly lower when administered by the oral versus IV route, exposure to the active phosphoramide mustard is equivalent between the two routes in dogs (Warry et al., 2011). Thus, doses for oral and IV administration are the same.

Clinical use: Cyclophosphamide is commonly used as part of multiagent protocols in the treatment of canine and feline lymphomas and as part of combination immunosuppressive therapy. Cyclophosphamide may also have utility in the treatment of canine malignant mammary gland tumors (Sorenmo, 2003). Dosing of cyclophosphamide in dogs ranges from 250 mg/m^2 IV or oral bolus, to a more fractionated schedule of 50 mg/m^2 daily for 3 days on a 3-week cycle (Lori et al., 2010). Low-dose cyclophosphamide (10–15 mg/m^2/day) has also been investigated in solid tumors of dogs as part of a metronomic therapy protocol and has demonstrated some efficacy in that setting (Elmslie et al., 2008; Burton et al., 2011). Cyclophosphamide is available as 25- and 50-mg tablets and as a 25-mg/ml injection. Tablets should not be broken or split prior to administration.

Ifosfamide

Ifosfamide (isophosphamide) (Figure 44.3) is a structural isomer of cyclophosphamide that, like cyclophosphamide, requires metabolic activation by hepatic P450 enzymes to form the bifunctional alkylating compound isofosforamide mustard (Creaven et al., 1974). The structural differences between cyclophosphamide and ifosfamide result in a reduced rate of the initial metabolism of ifosfamide to the active form. In addition, a secondary pathway of metabolism that generates chloroacetaldehyde results in the requirement of larger doses of ifosfamide when compared to cyclophosphamide (Wagner, 1994). Although ifosfamide is mostly indicated for treatment of sarcomas in animals, it has a relatively broad

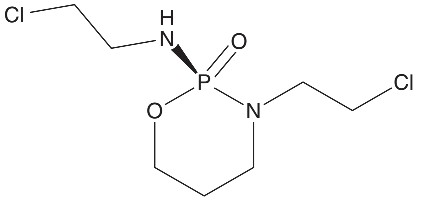


Figure 44.3 The nitrogen mustard alkylating agent ifosfamide, a structural isomer of cyclophosphamide.

range of antitumor activity and has been evaluated in both canine and feline neoplasms. In dogs, a dose of 350 to 375 mg/m^2 has been evaluated in a variety of tumor types with DLT of neutropenia occurring 7 days post treatment (Rassnick et al., 2000). In cats, the recommended starting dose of ifosfamide is 900 mg/m^2 and has been evaluated in the treatment of vaccine-associated sarcoma with an overall response rate of 41% (Rassnick et al., 2006). The dose-limiting toxicity in cats is also neutropenia and the majority of cats tolerate ifosfamide treatment. Nephrotoxicosis is a potential adverse effect in cats treated with ifosfamide. Prophylactic therapy with saline diuresis and MESNA appear to be effective means to prevent sterile hemorrhagic cystitis following ifosfamide treatment and diuresis should be considered to reduce the risk of nephrotoxicosis in cats. The reason for the difference in the dose tolerated by dogs and cats has not been systematically evaluated but is likely to involve species differences in metabolism with regard to exposure to active and/or toxic metabolites. There is evidence of autoinduction of ifosfamide metabolism in humans with multiple fractionated doses resulting in increased levels of metabolites, which may result in increased toxicity (Kurowski et al., 1991; Kaijser et al., 1994), but this has not been evaluated in veterinary patients. As with most cytotoxic drugs, ifosfamide is most likely to provide the greatest benefit when used as part of a combination protocol. Ifosfamide is available in 1 or 3-gram vials for reconstitution and intravenous administration

Mechlorethamine

Mechlorethamine (mustargen), sometimes referred to as “nitrogen mustard,” was the first alkylating agent used clinically (Figure 44.4). Mechlorethamine is a bifunctional alkylating agent that is capable of forming interstrand DNA cross-links as a mechanism of cytotoxicity (Kohn et al., 1966). Unlike cyclophosphamide and ifosfamide, mechlorethamine undergoes spontaneous hydrolysis to form active nucleophilic compounds. Thus, interactions with drugs that inhibit or induce hepatic metabolism are not anticipated with mechlorethamine. Entry of mechlorethamine into cells appears to involve a carrier-mediated, active process and resistant cells demonstrate reduced entry of drug into the cell (Goldenberg et al., 1970). Structural analogs of mechlorethamine, such as cyclophosphamide, melphalan, and chlorambucil, do not appear to inhibit cellular uptake of mechlorethamine. In veterinary medicine, mechlorethamine is most commonly used in the treatment of relapsed or resistant multicentric canine lymphoma as part of the MOPP (mustargen, vincristine, procarbazine, prednisone) protocol (Rassnick et al., 2002; Brodsky et al., 2009; Northrup et al., 2009). Myelosuppression and gastrointestinal toxicity are the dose-limiting toxicities reported for mechlorethamine in combination protocols. The most commonly reported dose for mechlorethamine in dogs when used in combination protocols in 3 mg/m^2 on days 0 and 7 of a 21-day cycle. Mechlorethamine is available as a lyophilized powder in 10-mg vials.

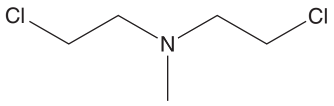


Figure 44.4 Mechlorethamine, or mustargen, sometimes referred to as “nitrogen mustard” was the first alkylating agent to be used clinically.

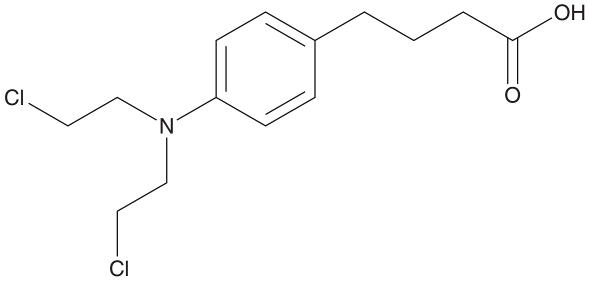


Figure 44.5 Chlorambucil, an orally bioavailable nitrogen mustard alkylating agent.

Chlorambucil

Chlorambucil (leukeran) (Figure 44.5) is an orally bioavailable alkylating agent that, along with the metabolite phenylacetic acid mustard, has direct bifunctional alkylating ability (Mitoma et al., 1977; Goodman et al., 1982). Chlorambucil is most often used in slower growing neoplasia such as chronic lymphocytic leukemia (CLL) but has been used in other types of cancers such as lymphoma and mast cell tumor (Taylor et al., 2009; Campbell et al., 2013). In cats, chlorambucil has been used in the treatment of GI lymphoma in combination with glucocorticoids, where it demonstrated favorable efficacy and was well tolerated (Stein et al., 2010). Metronomic therapy with chlorambucil has also been reported for a variety of neoplasms in dogs and may be a treatment option for dogs with transitional cell carcinoma of the urinary bladder (Leach et al., 2012; Schrempp et al., 2013). Chlorambucil has also been utilized as an alternative to cyclophosphamide when the latter has resulted in the development of sterile hemorrhagic cystitis. Chronic oral dosing in dogs with CLL generally begins with 3 to 6 mg/m^2 daily for 1 to 2 weeks followed by a reduction to 3 to 6 mg/m^2 every other day. Metronomic dosing studies have used a daily dose of 4 mg/m^2 in dogs while a bolus dosing regimen of 20 mg/m^2 every 2 weeks has been evaluated and found to be well tolerated in cats with GI lymphoma. The major dose-limiting toxicity for

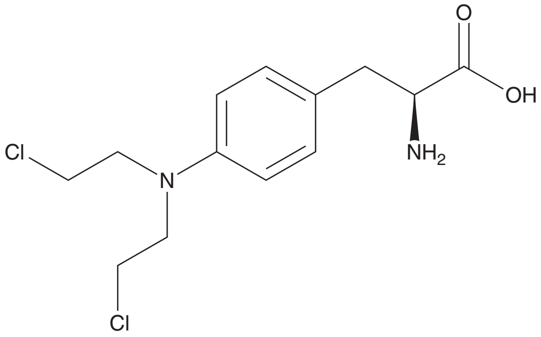


Figure 44.6 Melphalan, structurally similar to chlorambucil, is charged and hydrophilic and requires active uptake into cells by amino acid transporters.

chlorambucil is myelosuppression with neutropenia and a lesser degree of thrombocytopenia. In humans, myoclonus has been reported as a rare side effect following both high- and low-dose treatment and myoclonus following chlorambucil treatment has also been reported in a cat (Benitah et al., 2003). Chlorambucil is available as 2-mg tablets for oral administration. Tablets should not be broken and animal doses should be adjusted to the nearest whole tablet.

Melphalan

Melphalan (alkeran) is a nitrogen mustard-containing alkylating agent (L-phenylalanine mustard) that has direct DNA alkylating properties (Figure 44.6). Structurally, melphalan is similar to chlorambucil yet it is charged and hydrophilic at neutral pH and thus requires active uptake by amino acid transporters, which can be blocked by the amino acid leucine (Del Amo et al., 2008). Melphalan is orally bioavailable with a relatively large percentage eliminated unchanged in the urine in humans (Tew et al., 2001). Melphalan is most commonly used for the treatment of multiple myeloma in dogs and cats where initial positive responses are frequent, although complete eradication of all neoplastic myeloma cells is unlikely and eventual relapse is anticipated (Matus et al., 1986). Melphalan has also been used as a rescue therapy for relapsed lymphoma in dogs (Alvarez et al., 2006; Back et al., 2013). Myelosuppression and, in particular, a delayed thrombocytopenia is the major dose-limiting toxicity for melphalan and monitoring of complete blood count (CBC), including platelet counts, should be performed every 2 weeks for the first 2 months of therapy, then monthly thereafter. Melphalan therapy appears to be more myelosuppressive in cats than in dogs (Hanna, 2005). Initial dosing of melphalan is 0.1–0.2 mg/kg by mouth daily for 7–10 days followed by a reduction to 0.05 mg/kg daily, continuously. Alternatively, a pulse-dosing regimen of 7 mg/m^2 by mouth, daily for 5 days every 3 weeks has been utilized and is well-tolerated by dogs. In cats, long-term therapy consists of 0.1 mg/kg by mouth once every 7 days, continuously (Vail, 2013). Melphalan is available for oral use as 2-mg tablets and for intravenous injection as a 50-mg vial.

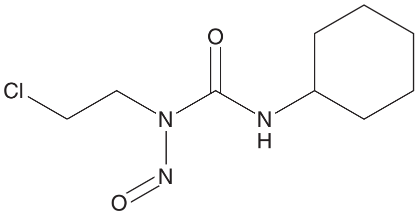


Figure 44.7 Structure of the lomustine, or CCNU, belonging to the nitrosurea class of alkylating agents.

Nitrosoureas

Cyclohexylchloroethylnitrosourea (lomustine)

Cyclohexylchloroethylnitrosurea (CCNU) is a monofunctional alkylating agent with a single chloroethyl group (Figure 44.7) that is capable of producing DNA–DNA and DNA–protein crosslinks as a mechanism of cytotoxicity (Kohn, 1977). CCNU is extremely lipophilic and capable of crossing the blood–brain barrier and thus has been used to treat neoplasia of the central nervous system. The main indications for use of CCNU in veterinary medicine are in brain tumors, histiocytic sarcoma, mast cell tumors, and epitheliotropic, and relapsed or refractory lymphomas (Fulton and Steinberg, 1990; Moore et al., 1999; Rassnick et al., 1999; Williams et al., 2006; Skorupski et al., 2007; Saba et al., 2009). CCNU is extensively metabolized in the liver with a large "first pass" effect resulting in undetectable plasma levels of the parent compound and rapid appearance of the active monohydroxylated metabolites (Lee et al., 1985). In both cats and dogs, CCNU has delayed and cumulative effects on the bone marrow causing neutropenia and thrombocytopenia with a nadir that can range from 7 to 28 days, and often has a prolonged recovery period (Moore et al., 1999; Rassnick et al., 2001). The extensive hepatic metabolism that occurs may also be partly responsible for the development of hepatotoxicity with chronic use, requiring dose reduction or complete cessation of lomustine therapy (Henry et al., 1973; Heading et al., 2011). Increases in hepatic enzymes occur in almost 90% of dogs receiving CCNU but the frequency and severity of hepatic enzyme elevation may be reduced by the concurrent administration of denamarin, a drug containing *S*-adenosylmethionine and sylibinin, which have both been evaluated for various types of hepatopathy in dogs (Skorupski et al., 2011). The most common dosing regimens for CCNU are 70–80 mg/m^2 PO every 3 weeks in dogs and 50–60 mg/m^2 PO every 4–6 weeks in cats. Lomustine is available as 10, 40, and 100-mg capsules.

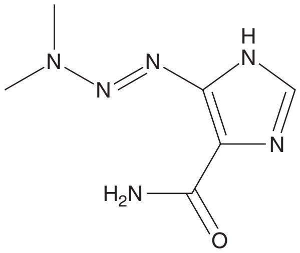


Figure 44.8 Dacarbazine, an imidazole-carboxamide derivative alkylating agent.

Other Alkylating Agents

Dacarbazine

Dacarbazine (DTIC) is an imidazole–carboxamide derivative that is structurally related to purines (Figure 44.8) and its development was initially an attempt to generate an antimetabolite that would inhibit purine synthesis. However, the principle mechanism of action of this drug is to induce methyl adducts to DNA and the formation of O-6 methylguanine is the main lesion responsible for the cytotoxicity (and mutagenic) effect of DTIC (Marchesi et al., 2007). DTIC is a non cell cycle-specific drug that is active in all phases of the cell cycle. Like cyclophosphamide and ifosfamide, dacarbazine requires metabolic activation by hepatic cytochrome P450 enzymes to form the active species 5-[3-methyl-triazen-1-yl]-imidazole-4-carboxamide (MTIC) and the relative activity as an effective anticancer drug may depend upon the level of P450 activity (Reid et al., 1999). Coadministration of drugs that alter P450 activity can thus be anticipated to result in important drug–drug interactions that can reduce activity or enhance toxicity of dacarbazine. Administration to cats has not been evaluated in terms of their ability to convert the parent drug to the active metabolite, and thus use in cats is not recommended. The main dose-limiting toxicity of DTIC is gastrointestinal and generally presents as vomiting and diarrhea. In addition, a delayed myelosuppression may occur at 2 to 3 weeks postadministration and can be severe in some cases. Thrombocytopenia may also be encountered 1 to 2 weeks following therapy and may result in treatment delays (Griessmayr et al., 2009). Dacarbazine has poor oral bioavailability and is given by the intravenous route, and renal tubular secretion appears to be the major elimination route; dose adjustments are recommended for patients with renal or hepatic disease. DTIC does not cross the blood–brain barrier effectively and thus is not used for intracranial tumors. In veterinary medicine DTIC has mainly been used in the treatment of relapsed or resistant canine lymphoma (Van Vechten et al., 1990; Flory et al., 2008; Griessmayr et al., 2009) and in advanced-stage hemangiosarcoma (Dervisis et al., 2011).

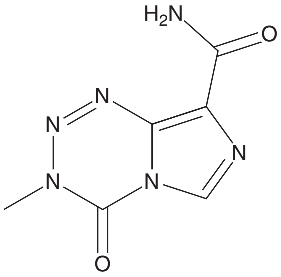


Figure 44.9 Temozolamide is an orally bioavailable alkylating agent related to dacarbazine; it has better blood–brain barrier penetration making it useful for CNS neoplasia.

The dose of dacarbazine ranges from 800–1,000 mg/m^2 every 2–3 weeks as a single agent to 600 mg/m^2 IV when used in combination protocols. Dacarbazine is generally administered as a 4 to 5-hour infusion in 5% dextrose solution or normal saline, as more rapid administration can result in severe phlebitis. Dacarbazine is available as a lyophilized powder in 100 or 200-mg vials.

Temozolomide (temodar)

Temozolomide (Figure 44.9) is an alkylating agent that is related to dacarbazine. It is a prodrug whose active metabolite is the same as dacarbazine (MTIC); however, there are important differences with regard to the mode of activation and pharmacokinetics of temozolomide that make it distinct from dacarbazine in its clinical applications. Temozolomide does not require hepatic activation by P450 enzymes but is spontaneously hydrolyzed at physiological pH to form MTIC (Marchesi et al., 2007). Additionally, temozolomide is orally bioavailable and is capable of crossing the blood–brain barrier as the parent compound, which can then be converted to the active MTIC species within brain tissues making it useful for CNS neoplasia (Marchesi et al., 2007). Temozolomide has been evaluated in clinical trials of canine cancer and was compared to dacarbazine for relapsed or refractory lymphoma, where the responses were similar in terms of rate and duration but temozolomide caused less hematological toxicity and was more convenient to administer (Dervisis et al., 2007). The dose of temozolomide evaluated in this study ranged from 60 to 100 mg/m^2 PO daily for 5 days on a 3-week cycle. Temozolomide is available as 5, 20, 100, 140, 180, and 250-mg capsules.

Procarbazine

Procarbazine (PCB) (Figure 44.10) is another prodrug alkylating agent that does not have cytotoxic effects in cells without chemical or metabolic activation (Gale et al., 1967). Additionally, the exact mechanism of cytotoxicity by procarbazine is not fully understood; however, evidence exists that sensitivity to procarbazine is associated with reduced cellular levels of the enzyme

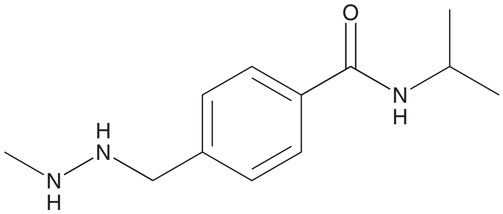


Figure 44.10 Procarbazine

responsible for repair of O-6 methylguanine adducts, inferring methylation of guanine as a mechanism of action (Schold et al., 1989). Procarbazine is rapidly and completely absorbed after oral dosing and the metabolites appear rapidly in plasma following oral dosing. Procarbazine appears to cross the blood–brain barrier in dogs and there is evidence for autoinduction of metabolism of procarbazine with metabolite levels increasing after multiple daily doses, which correlate with increased antitumor activity experimentally (Oliverio et al., 1964; Shiba and Weinkam, 1982, 1983). Procarbazine is also an inhibitor of monoamine oxidase (MAO) and drug–drug interactions may be expected with coadministration of sympathomimetics, tricyclic antidepressants, or phenothiazines, as well as with drugs that inhibit hepatic P450 enzymes such as cimetidine (Friedman et al., 2001). Procarbazine is generally used as part of combination therapy protocols for relapsed lymphoma in dogs (Rassnick et al., 2002, 2010; Brodsky et al., 2009; Northrup et al., 2009). The recommended dose is 50 mg/m^2 PO daily for 14 days of a 28-day cycle, although alternate-day dosing may be required due to limitations of tablet size for some dogs. Procarbazine is available in 50-mg capsules for oral administration.

Antitumor Antibiotics

Doxorubicin (Adriamycin)

Doxorubicin (Figure 44.11) is an anthracycline antibiotic that that was first isolated from *Streptomyces peucetius* var. *caesius* (Arcamone et al., 1969). Doxorubicin exhibits pleiotropic antitumor activity with evidence for cytotoxicity from direct distortion of the DNA double-helix through intercalation (Neidle, 1979), free radical formation through redox activity and reduction to the semiquinone (Davies and Doroshow, 1986), inhibition of DNA and RNA polymerases (Zunino et al., 1975), inhibition of the activity of DNA topoisomerase II (Tewey et al., 1984), alteration in intracellular calcium homeostasis (Oakes et al., 1990), lipid peroxidation and alterations in cell membrane functions (Tritton, 1991), and inhibition of thioredoxin reductase resulting in reduced activity of ribonucleotide reductase (Mau and Powis, 1992). While each of the putative actions have been implicated in the efficacy and toxicity of doxorubicin, with the relative contribution of each still under debate, it is known that the majority of doxorubicin is found within the nucleus and intercalated into the DNA double helix. In addition, there does appear to be some degree of base recognition and sequence specificity to the intercalation of doxorubicin (Trist and Phillips, 1989). The cellular pharmacology of doxorubicin is characterized by extensive intracellular accumulation where it binds to DNA, associates with membranes, and is stored in various cellular compartments. Classically, doxorubicin has been classified as a topoisomerase II inhibitor and the reduced sensitivity seen in cells that have down-regulated topoisomerase II underscores the relative importance of that interaction for antitumor activity. The topoisomerase enzymes are ubiquitous nuclear enzymes that are responsible for relaxing supercoiled double-stranded DNA to allow for DNA replication and RNA transcription. The torsional strain on DNA is relieved by formation of a single-stranded nick (topoisomerase I) or a double-stranded nick (topoisomerase II) followed by passage of DNA through the nick and religation. The topoisomerase inhibitors bind to and stabilize the DNA/topoisomerase complex which prevents religation and ultimately leads to lethal double strand breaks. As discussed in Section Tumor Cell Sensitivity, resistance to doxorubicin may also be due to the classic MDR phenotype resulting from up-regulation of MDR1 and other ABC transporters.

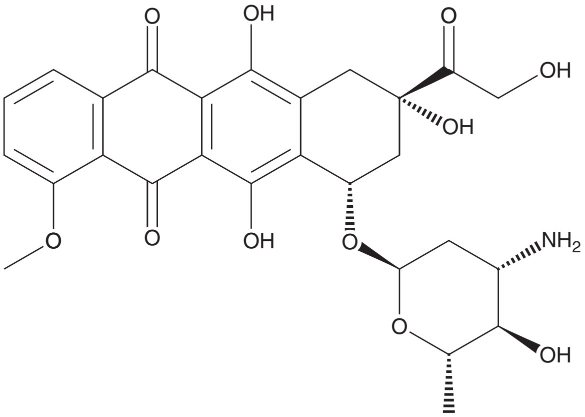


Figure 44.11 Structure of the antitumor antibiotic doxorubicin (Adriamycin). Doxorubicin in one of the most commonly used chemotherapeutic agents in veterinary oncology.

Doxorubicin is poorly bioavailable and is administered by the intravenous route. Following intravenous administration in dogs, doxorubicin concentrations drop rapidly in plasma as the drug distributes extensively to tissues, with the extent of distribution to specific tissues suggested to be determined by tissue DNA content (Terasaki et al., 1982). In dogs, a three-compartment model was shown to fit doxorubicin kinetics with half-lives of the α, β, and γ phases of 0.04, 0.44, and 19.8 hours, respectively

(Gustafson et al., 2002). Clearance in dogs is reported to be 46.5 l/h and steady-state volume of distribution is approximately 500 liters (Gustafson et al., 2002). In both humans and dogs, however, there is substantial interpatient variability in the pharmacokinetics of doxorubicin, leading to variability in toxic effects such that dose reductions are not uncommon following the first treatment. Elimination of doxorubicin occurs through renal and biliary excretion of parent drug and through metabolism to doxorubicinol, via aldo-keto reductases, and 7-hydroxy aglycone, via reductive cleavage in both hepatic and extrahepatic tissues (Ahmed et al., 1981).

The dose-limiting toxicities for doxorubicin include myelosuppression, gastrointestinal toxicity, and a well-established cumulative cardiotoxicity that places a limit the lifetime exposure in patients. For dogs, this lifetime limit is often set at 150 mg/m^2, which provides approximately five treatment cycles. This limit may be increased as dictated by a need for additional therapy such as with relapsed lymphoma after careful cardiac evaluation. Formation of free radicals and iron accumulation in cardiac mitochondria appear to underlie the development of congestive heart failure, and cardiac function should be carefully evaluated prior to initiation of therapy as well as prior to each treatment, particularly in at-risk breeds such as Boxers and Doberman Pinschers. During the course of treatment, any changes in cardiac function, such as the development of new murmurs, arrhythmias, or pulse deficits, should be pursued with a full diagnostic evaluation.

The administration of the iron-chelating agent dexrazoxane (Zinecard) has been shown in human patients to reduce the risk for cardiotoxicity and is increasingly used for this purpose in veterinary medicine Dexrazoxane has also been used to reduce the local tissue damage from extravasation of doxorubicin during administration. Doxorubicin causes some of the most severe local tissue necrosis following extravasation and this can result in a requirement for surgical debridement or amputation. Thus, it is critical that a carefully placed catheter be used for doxorubicin administration. Sedation may be required in some patients to ensure that a catheter is placed appropriately. If the catheter is not placed on the first stick, another vein should be chosen for use and treatment delays may be required to ensure that catheter placement is adequate. Cats administered doxorubicin often may not display the overt signs of cardiac toxicity that are seen in dogs but may develop renal tubular damage following repeated dosing, and monitoring of serum creatinine and urine specific gravity are recommended during therapy (O'Keefe et al., 1993). Doxorubicin produces poikilocytosis in cats (O'Keefe and Schaeffer, 1992). Doxorubicin can also cause acute anaphylactic reactions during administration due to histamine release; this often results in stopping the infusion and treatment with antihistamines and/or corticosteroids. In some situations a clinician may choose not to continue administration while some will continue but at a slower infusion rate. Slower rates of infusion are associated with reduced incidence of acute cardiac toxicity that can be seen with bolus dosing.

Standard dosing of doxorubicin in dogs is 30 mg/m^2, diluted in 50–100 ml of saline and given intravenously over 20–30 minutes every 3 weeks, with the slower infusions resulting in less risk for acute adverse reactions. In dogs that are less than 15 kg and in all cats, the dose is 1 mg/kg intravenously every 3 weeks. This difference in dosing is due to the fact that using the body surface area prescription base in small dogs and cats seems to lead to a relative overdose and increased toxicity. Doxorubicin has a very broad spectrum of antitumor activity and is used as a single agent and as part of many combination protocols for canine and feline lymphoma, osteosarcoma, and most neoplasms of mesenchymal (sarcoma) and epithelial (carcinoma) origin. Doxorubicin is available as a 2 mg/ml injectable solution. Other formulations of doxorubicin are available such as a pegylated, liposome-encapsulated form (Doxil) that has been demonstrated to be less cardiotoxic (Working et al., 1999) and has been evaluated in a few canine trials (Vail et al., 1997; Sorenmo et al., 2007; Teske et al., 2011).

Mitoxantrone

Mitoxantrone is an anthracenedione drug (Figure 44.12) that was developed in an effort to find a less toxic alternative to the structurally related anthracyclines (DOX). Mitoxantrone maintains a similar spectrum of cytotoxic mechanisms to doxorubicin, including DNA intercalation, inhibition of DNA and RNA polymerases, and inhibition of topoisomerase II (Foye et al., 1982; Crespi et al., 1986). However, mitoxantrone does not possess the same ability to induce cardiac dysfunction or produce local tissue necrosis if extravasated. This is due to the reduced ability to undergo one-electron reduction and generate free radicals as well as a lack of lipid peroxidation in cardiac myocytes (Patterson et al., 1983; Nguyen and Gutierrez, 1990). This results in mitoxantrone being used in some cases as a substitute for doxorubicin, although the spectrum of anticancer activity is narrower than that of doxorubicin. Following intravenous administration there is rapid and extensive distribution to tissues with an apparent volume of distribution of 26 l/kg and a long persistence of drug residues in tissues, with approximately 30% of the drug eliminated unchanged in urine and feces (Lu et al., 1984). The dose-limiting toxicities include gastrointestinal disturbance and myelosuppression; cardiac toxicity has not been reported in dogs. Resistance to mitoxantrone can be a result of increased drug efflux from up-regulation of MDR1, MRP, or BCRP as well as

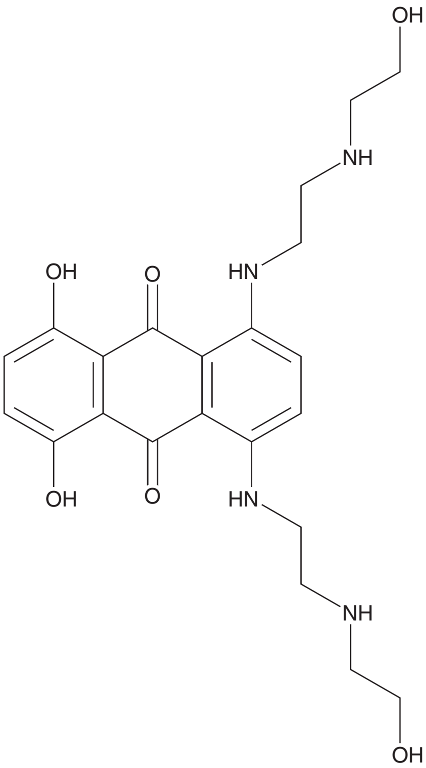


Figure 44.12 Mitoxantrone, an anthracenedione chemotherapy agent, with a similar spectrum of activity to doxorubicin and a lower likelihood of cardiac toxicity.

through alterations in topoisomerase II function (Hazlehurst et al., 1999).

Dosing is generally instituted at 5–6 mg/m^2 every 3 weeks as a slow intravenous bolus, and indications include lymphoproliferative disorders (lymphomas and leukemias) and potentially transitional cell carcinoma of the urinary bladder. Mitoxantrone may be chosen as a maintenance therapy for lymphoma in dogs that have reached their cumulative maximum dose of doxorubicin or in dogs at high risk for cardiotoxicity from doxorubicin. Mitoxantrone is available as a 2 mg/ml injectable formulation.

Actinomycin D

Actinomycin D (Dactinomycin; DACT) (Figure 44.13) is an actinomycin antibiotic produced by various species of *Streptomyces*. DACT interacts with both single and double-stranded DNA in a sequence-specific manner (gaunine–cytidine base pairs) and results in a potent inhibition of transcription and RNA translation, which block protein synthesis (Kawamata and Imanishi, 1960). DACT is not orally bioavailable and is administered intravenously. Following IV dosing in dogs there is a rapid fall in plasma drug concentrations with a concomitant accumulation of drug in peripheral tissues. DACT resides in tissues for an extended period of time with a tissue half-life of 47 hours in the dog (Galbraith and Mellett, 1975). Elimination of DACT is via biliary and urinary routes and there appears to be minimal metabolism (Galbraith and Mellett, 1975). Cross-resistance to anthracyclines and mitoxantrone has been demonstrated, implicating MDR1 up-regulation and drug efflux as a resistance mechanism to DACT.

Dose-limiting toxicities for actinomycin-D are myelosuppression and gastrointestinal disturbances. DACT is also capable of producing tissue necrosis if extravasation occurs during administration and thus care must be taken during placement of catheters. Actinomycin D is a radiation sensitizer by virtue of the inhibition of repair of sublethal radiation-induced DNA damage (D'Angio et al., 1959). DACT administration can also result in a phenomenon known as radiation recall where patients receiving DACT will experience inflammatory reactions in previously irradiated sites. Administration of corticosteroids may lessen these effects.

Actinomycin D is often used as part of combination protocols in the setting of relapsed lymphoma in dogs, or as a doxorubicin substitute in dogs with preexisting cardiac dysfunction. The standard dose is 0.5–0.75 mg/m^2 IV every 3 weeks and is available in 0.5-mg vials.

Antimetabolites

The antimetabolites are drugs that interfere with normal cellular functions, such as synthesis of DNA, by serving as false substrates for normal biochemical processes involved the synthesis of nucleotides or inhibiting further extension of the new DNA strand, leading to the inhibition of cell division. The antimetabolite class of drugs are cell-cycle specific and their toxic effects reflect activities in proliferating cells such as bone marrow and the gastrointestinal tract. These drugs do not directly bind to DNA, and thus are not associated with the late effects of secondary malignancies like the alkylating agents.

Methotrexate

Methotrexate (MTX) (Figures 44.14 and 44.15) is a folate analog that inhibits the synthesis of purine nucleotides. Reduced folate is required in the biosynthesis of purines and the cellular generation of reduced folate is dependent upon the enzyme dihydrofolate reductase (DHFR). Methotrexate competitively binds and inhibits DHFR, preventing the formation of reduced folate; this is the major mechanism of antitumor action of this drug (Bertino, 1963). Intracellular metabolism of methotrexate leads to the addition of glutamic acid residues to the drug (polyglutamation) and these polyglutamates act as direct inhibitors of folate-dependent enzymes that function in purine and thymidylate synthesis (Fabre et al., 1984; Allegra et al., 1985). Methotrexate entry into cells is active and depends on the function of the reduced

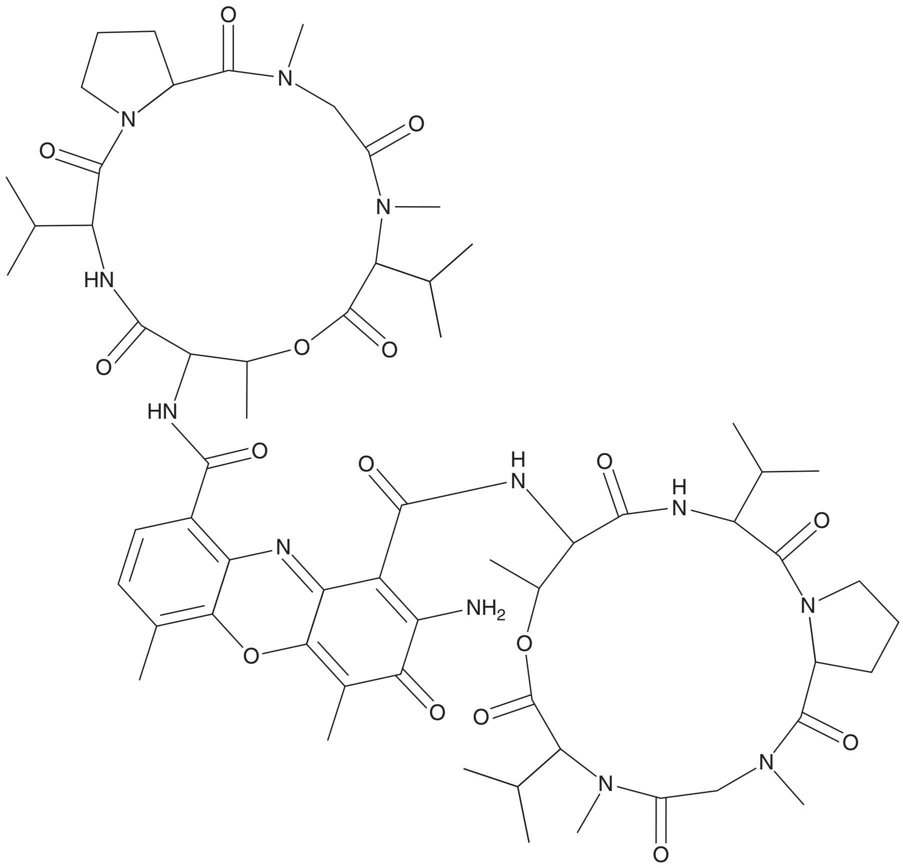


Figure 44.13 Structure of the antitumor antibiotic actinomycin D (dactinomycin).

folate carrier. Oral absorption of methotrexate is almost complete at lower doses but is reduced at high doses, suggesting saturation of absorption processes. MTX also undergoes enterohepatic cycling with some metabolism by intestinal bacteria (Wan et al., 1974). This enterohepatic cycling leads to gastrointestinal toxicity becoming evident at doses that do not produce significant myelosuppression. At higher doses, myelosuppression is also a dose-limiting toxicity with methotrexate. In humans, high-dose regimens are utilized and the myelosuppression is "rescued" by the administration of a source of reduced folate (Leucovorin). This drug has also been used in the prevention of toxicity following accidental ingestion of methotrexate in dogs (Lewis et al., 2010). Methotrexate has found limited use in veterinary medicine as a part of combination protocols for lymphoma. It is available as 2.5-mg tablets for oral administration and as 20, 50, 100, 200, 250-mg and 1-g vials for injection.

Cytosine Arabinoside (Cytarabine)

Cytosine arabinoside (Ara-C) is an analog of the purine nucleotide deoxycytidine, differing only by the presence of a hydroxyl group on the sugar so that it becomes arabinose instead of deoxyribose (Figure 44.16). The main effects of Ara-C in tumor cells are inhibition of DNA polymerase, which impedes DNA replication and repair (Furth and Cohen, 1968), and, more importantly, incorporation into DNA, which is the main mechanism of cytotoxicity for this agent (Kufe et al., 1980). In fact, there

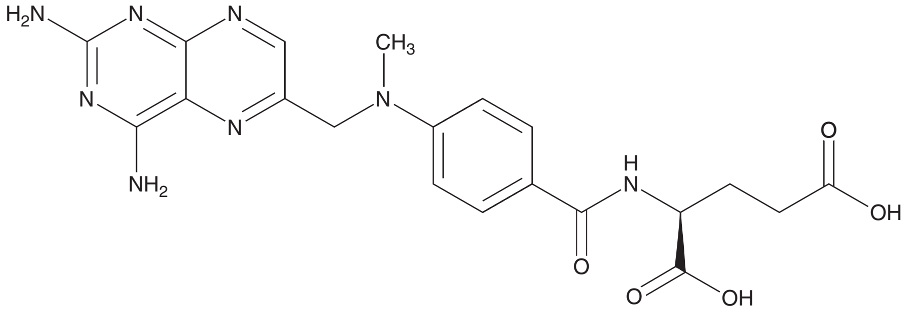


Figure 44.14 Structure of the antimetabolite and folic acid analogue methotrexate.

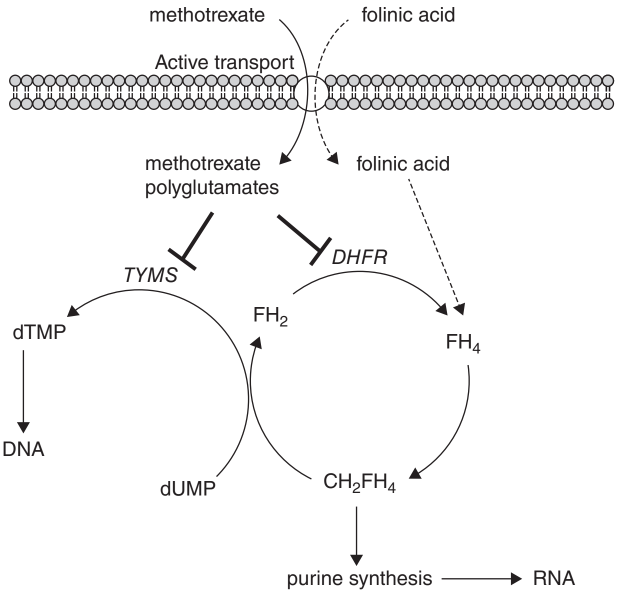


Figure 44.15 Mechanism of action for methotrexate. Methotrexate is actively transported into the cell by the reduced folate carrier. Formation of intracellular methotrexate-polyglutamates results in increased intracellular retention of drug. Methotrexate inhibits the activity of the dihydrofolate reductase enzyme (DHFR), preventing the conversion of dihydrofolate (FH_2) to tetrahydrafolate (FH_4) and blocking the synthesis of purine nucleotides. Methotrexate also functions to block the conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (dTMP), a reaction catalyzed by the enzyme thymidylate synthase (TYMS) and requiring reduced folate. Administration of exogenous sources of reduced folate (such as folinic acid, or Leucovorin) can reverse the cytotoxicity of methotrexate.

is a linear relationship between the amount of Ara-C incorporated into DNA and tumor cell survival (Kufe and Spriggs, 1985). Once Ara-C is incorporated into DNA, tumor cells do not seem to be able to remove it and this inhibits template function and DNA strand elongation. Ara-C is a cell-cycle phase-specific drug with greatest activity against cells in S phase. As a nucleotide analog, Ara-C is actively transported into cells utilizing a shared transporter for physiological nucleotides, and at high concentrations the transport process can become saturated and further entry is via passive diffusion (Jamieson et al., 1990). Once inside the cell, a series of phosphorylation processes convert Ara-C into the active triphosphate form (Ara-CTP) by the activities of deoxycytidine kinase, deoxycytidine monophosphate (dCMP) kinase, and nucleoside diphosphate kinase (Garcia-Carbonero et al., 2001).

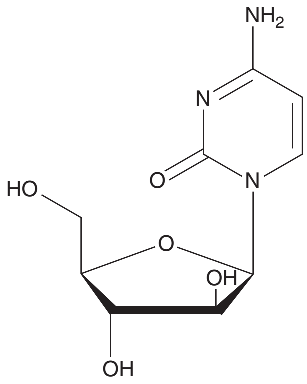


Figure 44.16 Cytosine arabinoside (cytarabine) is an analog of the purine nucleotide deoxycytidine.

Metabolism of Ara-C is primarily through deamination in hepatic and extraheptic tissues. The major dose-limiting toxicities of Ara-C are myelosuppression and, depending upon the dosing regimen used, gastrointestinal disturbances. Ara-C is water soluble and most often dosed by IV bolus, repeated subcutaneous injection, or IV infusion. Ara-C is capable of crossing the blood–brain barrier and is potentially useful in cases of intracranial lymphomas, although the route of administration may affect the overall efficacy as constant-rate infusion (CRI) dosing will maintain steady-state plasma concentrations longer, providing more exposure to the CNS than SQ dosing (Crook et al., 2013). The pharmacokinetics of Ara-C in dogs has been evaluated (Crook et al., 2013) and following SQ dosing, maximum plasma concentrations are achieved within 1 hour and the elimination half-life is 1.35 hours. Following initiation of a CRI, maximum plasma concentrations are achieved after 4 hours, at which point steady-state concentrations are maintained until termination of the CRI. Terminal half-life of Ara-C following CRI is similar to SQ at 1.2 hours. Ara-C is infrequently used in combination chemotherapy for lymphomas and leukemias, unless there is evidence of CNS involvement; however, a small study has demonstrated that there may be some improvement in canine patients with stage V lymphoma when Ara-C is administered in conjunction with the first two cycles of a standard CHOP-based (cyclophosphamide, doxorubicin, vincristine, prednisolone) combination protocol (Marconato et al., 2008a). CRIs of Ara-C over multiple days, as was done in the study above, may require bone marrow support with rhG-CSF and erythropoietin to avoid severe myelosuppression. Ara-C is more commonly used in the treatment of meningoencephalitis of unknown origin, often in combination with prednisolone. Cytosine arabinoside is available for IV or SQ injection in vials of 100 and 500 mg or 1 and 2 grams.

Gemcitabine

Gemcitabine (2′2′-difluorodeoxycytidine) (Figure 44.17) is a cytosine analog that is structurally similar to cytosine arabinoside but has a spectrum of activity that includes a variety of solid tumors. Gemcitabine entry into the cell is dependent upon carrier-mediated transport using nucleoside transporters (Mackey et al., 1998). Like

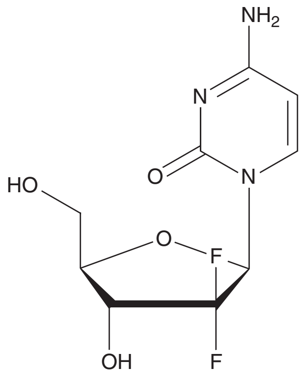


Figure 44.17 Chemical structure of gemcitabine.

Ara-C, gemcitabine must be metabolized inside the cell to the triphosphate derivative (dFdCTP), which then gets incorporated in DNA causing termination of strand elongation. Although gemcitabine is not as effective at termination of chain elongation as cytosine arabinoside, the long intracellular half-life of the triphosphate (up to 72 hours) provides a favorable pharmacokinetic profile with regard to tumor cell exposure (Plunkett et al., 1995). Additionally, the intermediate metabolites of gemcitabine have activities that enhance the overall growth inhibitory properties of the drug, such as inhibition of ribonucleotide reductase and stimulation of the enzyme responsible for activation (deoxycytidine kinase); thus gemcitabine appears unique in its “self-potentiation” capabilities (Plunkett et al., 1995).

Gemcitabine is administered by the intravenous route as it has low oral bioavailability, likely due to a high first-pass effect with hepatic conversion to the deaminated metabolite (dFdU), which also has some cytotoxic effects (Veltkamp et al., 2008). Gemcitabine is given as a slow infusion because this route leads to higher intracellular concentrations than bolus dosing. The pharmacokinetics of gemcitabine in dogs has been evaluated following infusion and bolus dosing and results demonstrate linear pharmacokinetics for the parent compound (Freise and Martin-Jimenez, 2006a, 2006b). After intravenous infusion, the maximum plasma concentration (C_{max}) occurred at 2 hours and gemcitabine clearance, steady-state volume of distribution, and half-life were 0.4 l/h*kg, 0.8 l/kg, and 1.5 hours, respectively (Freise and Martin-Jimenez, 2006b). Gemcitabine has been evaluated in a number of tumor types including osteosarcoma and a variety of carcinomas, often being combined with carboplatin (Marconato et al., 2008b, 2011; Dominguez et al., 2009; Elpiner et al., 2011; McMahon et al., 2011; De Brito Galvao et al., 2012). Gemcitabine has also been evaluated in cats in combination with carboplatin (Martinez-Ruzafa et al., 2009) or as a radiosensitizing agent (Jones et al., 2003). Doses of gemcitabine vary with the treatment protocol and the other agents used in conjunction; reported doses include 350–400 mg/m^2 weekly for 5 weeks; 800 mg/m^2 given over 60 minutes every 7 days; 625 mg/m^2 biweekly; and 2 mg/kg on days 1 and 8 of a 21-day cycle. In all cases, neutropenia appears to be the dose-limiting toxicity with gastrointestinal toxicity generally mild and self-limiting. The lack of clear benefit to gemcitabine therapy for any specific tumor type to date may suggest that further studies into the proper scheduling and dosing of this agent are required in veterinary medicine. Gemcitabine is available in 200 and 1000-mg vials of lyophilized powder.

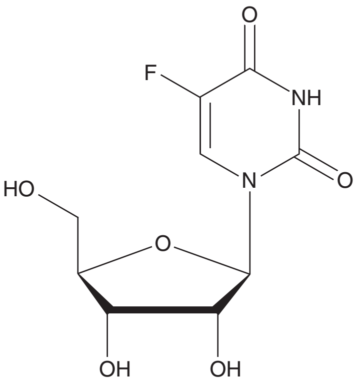


Figure 44.18 5-fluorouracil is a halogenated analog of uracil.

5-fluorouracil

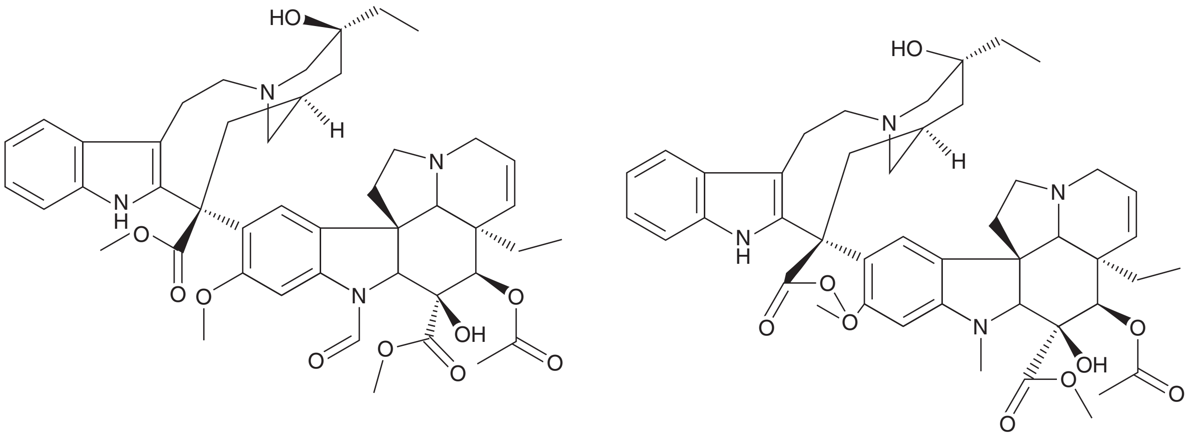
5-fluorouracil (5-FU) (Figure 44.18) is a halogenated analog of uracil that was the result of early attempts to design “targeted” agents in cancer therapy when it was noted that tumor cells utilize uracil more avidly than normal cells, providing a rational means by which neoplastic cells might be targeted (Rutman et al., 1954). 5-FU is actively transported into cells by a shared system that also transports uracil, adenine, and hypoxanthine (Wohlhueter et al., 1980). Similar to Ara-C and gemcitabine, 5-FU is converted enzymatically to active nucleotide forms intracellularly. The mechanisms by which 5-FU results in cytotoxicity include interference with RNA synthesis and function, inhibition of thymidylate synthetase leading to depletion of thymidine mono- and triphosphates, and incorporation into DNA reducing stability (Grem, 2001). In human oncology, 5-FU has found use in a number of solid tumor types including carcinomas of the colon and breast, and is often used in combination with other drugs or ionizing radiation. 5-FU is administered by the intravenous route and undergoes extensive hepatic and extrahepatic metabolism, primarily by the enzyme dihydropyrimidine dehydrogenase (DPD) (Kuan et al., 1998). In dogs, myelosuppression and gastrointestinal toxicity are commonly encountered. In both dogs and cats 5-FU causes neurotoxicity and, in cats, this is severe enough that use of the drug is contraindicated for the species (Harvey et al., 1977; Dorman et al., 1990; Hammer et al., 1994). Reported doses for systemic therapy in dogs are 150 mg/m^2 IV, weekly. Local or topical therapy is the more common route of administration in veterinary medicine as intraincisional therapy in spindle cell tumors of dogs and intralesional therapy for sarcoids in horses are reported to have some beneficial biological effect (Stewart et al., 2006; Marconato et al., 2007). 5-FU is available in 50 mg/ml vials.

Antimicrotubule Agents

Microtubules are principally recognized for their role in separating the duplicate set of chromosomes during mitosis, but they also play critical roles in cellular functions during interphase such as intracellular transport, maintenance of cell shape, neurotransmission, and relay of signals between the cell surface and the nucleus. Microtubules are composed of α- and β-tubulin dimers and a dynamic process of polymerization and depolymerization is required for proper microtubule function. Agents that are used clinically to inhibit microtubule function are structurally complex, naturally occurring or semisynthetic compounds that act to interfere with the normal dynamics of microtubule formation.

Vinca Alkaloids: Vincristine and Vinblastine

The vinca alkaloids vincristine (VCR) and vinblastine (VBL) (Figure 44.19) are naturally occurring plant alkaloids from the pink periwinkle plant (*Catharanthys roseus*). The main action of these drugs is to bind a distinct site on tubulin and prevent the formation of microtubules, thus disrupting the mitotic apparatus and resulting in metaphase arrest and cytotoxicity (Tucker et al., 1977; Wilson et al., 1982). The accumulation of mitotic figures in treated cells correlates with both the drug concentration and duration of treatment. Vinca alkaloids enter the cell via simple diffusion and are rapidly taken into cells. These drugs accumulate inside cells with intracellular concentrations of 150- to 500-fold more than extracellular concentrations; intracellular accumulation is greater for VCR than VBL. Resistance to the vinca alkaloids can occur through two distinct mechanisms. Vinca alkaloids are substrates for MDR1/P-glycoprotein and the classic multidrug resistant phenotype secondary to up-regulation of the transporter results in cross-resistance to anthracyclines (DOX), anthracenediones (mitoxantrone), and vinca alkaloids. Additionally, alterations in the binding site on tubulin molecules or increased expression of an alternate tubulin isoform with reduced affinity for the vinca alkaloids can result in reduced binding affinity and cellular resistance (Houghton et al., 1985; Ranganathan et al., 1998). The relative importance of tubulin alterations and expression of different tubulin isoforms to resistance in veterinary medicine has not been explored. The vinca alkaloids are administered by intravenous bolus injection and care must be taken to avoid extravasation, particularly with vincristine which can cause severe soft tissue injury. Following intravenous administration of vinca alkaloids there is a rapid decline in plasma concentration as drug distributes to peripheral tissues and intracellular spaces, followed by a slow elimination phase, primarily characterized by hepatic metabolism and biliary excretion of parent drug and metabolites. The spectrum of antitumor activity differs slightly between vincristine and vinblastine yet the principal toxicities vary dramatically. Vincristine is minimally myelosuppressive at standard doses and is a therapeutic option for neutropenic patients. In humans, a cumulative peripheral neurotoxicity is the principal toxicity following VCR administration, although central nervous system toxicity is possible



vincristine

vinblastine

Figure 44.19 Structures of the vinca alkaloid antitumor drugs vincristine (left) and vinblastine (right).

and has been documented in a dog with a mutation of the *MDR1* gene (Krugman et al., 2012). In contrast, vinblastine is less likely to produce neurotoxicity but is myelosuppressive with neutrophil nadirs occurring between 4 and 7 days after treatment.

Vincristine is most often used as a component of multiagent protocols for lymphoma and is curative in canine transmissible venereal tumor (CTVT) following three weekly doses (Ganguly et al., 2016). Vincristine has also been used to increase peripheral platelet numbers in cases of severe primary immune-mediated thrombocytopenia (IMT) in dogs (Rozanski et al., 2002). Vincristine is generally dosed at 0.5–0.75 mg/m^2 IV, weekly, or as defined by the specific protocol being used. For cases of IMT, the dose of vincristine is 0.02 mg/kg. Vinblastine is most often used in the treatment of canine mast cell tumors, either as a single agent or in combination (Rassnick et al., 2008). Vinblastine doses range from 2.5 to 3.5 mg/m^2 IV every 2 to 3 weeks (Bailey et al., 2008; Vickery et al., 2008). Both drugs are available as 1 mg/ml injectable solutions.

Taxanes

Paclitaxel

Paclitaxel (Figure 44.20) is an antimicrotubule agent that was originally isolated from the Pacific yew tree, *Taxus brevifolia*, in the early 1970s. By stabilizing microtubules and preventing depolymerization the taxanes disrupt normal tubulin dynamics and, similar to the vinca alkaloids, prevent normal chromosomal segregation during mitosis leading to cell death. In human oncology, paclitaxel has established activity in a variety of cancers, including breast cancer, where combinations utilizing paclitaxel have proven to increase survival rates (Simpson and Plosker, 2004). Because of poor solubility, paclitaxel is formulated in a Cremephor EL carrier, which is responsible for hypersensitivity reactions that are common following administration. In dogs, paclitaxel administration has resulted in partial responses in tumors such as osteosarcoma, mammary carcinoma, and malignant histiocytosis (Poirier et al., 2004). However, significant hypersensitivity reactions may still be seen in dogs due to the Cremephore EL carrier even with administration of antihistamines and corticosteroids, which limits its clinical utility. A Cremephore-free paclitaxel (Paccal® Vet CA-1), which is formulated in a water-soluble micellar form, has been evaluated in dogs and is associated with a greatly reduced incidence of hypersensitivity reactions and shows promising clinical benefit (Rivera et al., 2013). The suggested starting dose is 150 mg/m^2 intravenously every 21 days and at this dose the most common toxicities are associated with gastrointestinal toxicity and myelosuppression that are generally mild and manageable (Von Euler et al., 2013). A randomized clinical trial in dogs showed Paccal® Vet to be well tolerated and have superior efficacy compared to lomustine in nonresectable mast cell tumors (Vail et al., 2012).

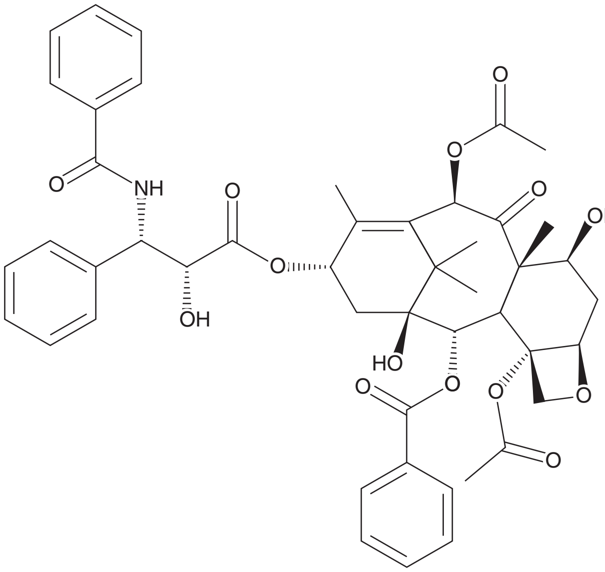


Figure 44.20 Structure of the antimicrotubule agent paclitaxel, originally isolated from the Pacific Yew tree.

The pharmacokinetics of Paccal® Vet has been determined following a single IV dose of 100–150 mg/m^2. A two-compartment model best fit the data with a short distribution phase and an elimination half-life of approximately 3.5 hours. In dogs, clearance values are widely variable but fall within the reported range of clearance values of paclitaxel in humans (Von Euler et al., 2013). Elimination of paclitaxel is primarily through hepatic metabolism via cytochrome P450 enzymes, and oral bioavailability is limited due to a significant first-pass effect and drug efflux by P-glycoprotein (Jibodh et al., 2013). Importantly, at the time of publication, Paccal® Vet CA-1 no longer has conditional approval and no legal marketing status; however, this may likely change in the future and the drug may become available once more, with a different dose recommendation.

Platinum Containing Agents

Cisplatin, carboplatin, and oxaliplatin are cytotoxic compounds that are based on elemental platinum. These drugs resemble alkylating agents in that they can form strong chemical bonds with nucleophiles such as the nitrogen in nucleic acids and sulfur in proteins. While platinum drugs are capable of binding to DNA, RNA, and proteins, the binding to DNA appears to correlate with drug-induced cell killing (Zwelling et al., 1979). All of the clinically useful platinum compounds are bifunctional and capable of producing a covalent cross-link between two nucleophilic atoms of a molecule. Generally,

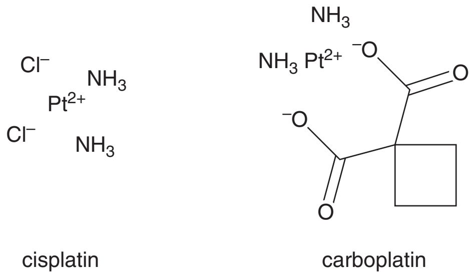


Figure 44.21 Structures of the platinum-containing antitumor agents cisplatin (left) and carboplatin (right).

if the two sites are on the same DNA strand, the lesion is referred to as a *DNA adduct* while *DNA cross-links* are formed by binding sites on different DNA strands. These DNA lesions inhibit DNA replication and transcription and ultimately induce apoptosis. Reactions with water, whereby the chloride groups of cisplatin or carboxylato groups of carboplatin are sequentially replaced with hydroxyl groups in a process termed *aquation*, are important components of the cellular pharmacology of platinum drugs with regard to transport across cell membranes and trapping within the cell. Resistance to the platinum agents can be through cytosolic inactivation of the drug secondary to glutathione conjugation or inactivation from other sulfhydryl-containing groups such as proteins. Increased repair of the DNA damage induced by platinum drugs is another mechanism of resistance and, specifically, increased nucleotide excision repair (NER) is associated with high-level resistance (Parker et al., 1991; Reed, 2001). Cisplatin and carboplatin (Figure 44.21) are both administered intravenously and there is extensive binding to plasma and tissue proteins. Elimination of cisplatin and carboplatin is essentially entirely by renal elimination of bound and unbound forms. There is a strong relationship between carboplatin exposure (AUC) and glomerular filtration rate in humans and cats, and dosing strategies that target a specific AUC based on patient glomerular filtration have been developed for both species (Calvert et al., 1989; Bailey et al., 2004). The pharmacokinetics of carboplatin in dogs has been reported previously (Page et al., 1993).

Cisplatin has been utilized for the treatment of squamous cell carcinoma, osteosarcoma, transitional cell carcinoma, and nasal adenocarcinoma (Knapp et al., 1988; Hahn et al., 1992). Nephrotoxicity is associated with cisplatin administration, which necessitates aggressive fluid diuresis prior to and immediately after drug administration. Standard protocols involve a 4-hour diuresis before, and an additional 2-hour diuresis with normal saline following, the IV bolus of cisplatin. Because of the significant time commitment involved with cisplatin therapy, carboplatin is commonly chosen instead. Additionally, emesis is common in cisplatin-treated patients and antiemetic therapy is commonly required. Doses of cisplatin are generally 50–70 mg/m^2 IV every 3 weeks. Cisplatin is contraindicated in cats due to the development of fulminant pulmonary edema (Knapp et al., 1987).

Carboplatin is often preferred over cisplatin because of reduce incidence of nausea/vomiting and nephrotoxicity. Myelosuppression is the dose-limiting toxicity with carboplatin and the neutrophil nadir typically occurs 14 days following administration. Carboplatin is commonly used in combination with doxorubicin for canine appendicular osteosarcoma as well as a variety of carcinomas. Doses reported for dogs and cats are 300 and 240 mg/m^2 IV every 3 weeks, respectively (Bacon et al., 2008; Kisseberth et al., 2008; Skorupski et al., 2016). As mentioned previously, AUC based dosing is possible in cats utilizing measured glomerular filtration and may provide a rational means of treating patients with preexisting renal dysfunction.

Miscellaneous Drugs

L-Asparaginase

The growth of all cells is dependent upon the availability of nutrients and cofactors that are required for protein synthesis. A long-desired goal of cancer therapy has been to identify differences between normal and neoplastic cells with regard to nutritional requirements that might be exploited. L-asparaginase is one example of a therapy that takes advantage of qualitative differences in the nutritional requirements of tumor cells. L-asparagine is a nonessential amino acid that in synthesized intracellularly by the enzyme L-asparagine synthetase; many tumor cells, and particularly those derived from the lymphoid lineage, are deficient in L-asparagine synthetase and must obtain asparagine by scavenging it from circulation (Haley et al., 1961). Circulating L-asparagine is hydrolyzed by L-asparaginase, producing L-aspartic acid and ammonia as end products and this depletes the tumor cells in L-asparagine, resulting in the induction of apoptosis (Story et al., 1993). The constitutive expression of L-asparagine synthetase in most normal tissues accounts for the modest toxicity seen with asparagine depletion from circulation.

L-asparaginase (Elspar) is derived from *E. coli* and can be administered either subcutaneously, intramuscularly, or intraperitoneally. In humans, the plasma half-life is approximately 30 days and elimination is via metabolic degradation; no pharmacokinetic studies specific to dogs were found.

Although most susceptible tumors will respond with a dramatic reduction in size, resistance to L-asparaginase develops rapidly and most likely reflects outgrowth of populations of tumor cells that have functional L-aspargine synthetase. Side-effects of L-asparaginase administration are not extremely common in dogs but may involve hypersensitivity reactions, particularly with repeated administration of this foreign protein, which can be managed with antihistamines and/or corticosteroids. Other toxicities that are rarer are associated with reduced protein synthesis from reduced asparagine pools; reduce protein synthesis in the liver can result in depleted clotting factors but clinically significant bleeding or thrombosis in dogs following L-asparaginase is extremely rare. Pancreatitis following L-aparaginase has been recognized in the dog (Schleis et al., 2011) and is better characterized in children (Raja et al., 2012). As one of the end products of asparagine hydrolysis is ammonia, a clinically significant hyperammonemia may develop following therapy and, particularly in patients with preexisting hepatic disease, this may result in a syndrome that resembles ammonia encephalopathy (Lyles et al., 2011).

L-asparaginase is utilized exclusively in the treatment of lymphoid malignancies in combination protocols to induce remission or in the treatment of relapsed or refractory lymphoma (Valerius et al., 1997; Saba et al., 2007). There is evidence suggesting that L-asparaginase may best be utilized in the setting of relapsed or refractory disease as time to remission and duration of first remission in treatment-naïve dogs was not significantly different when protocols with and without L-asparaginase were compared (MacDonald et al., 2005).

The dose of L-asparaginase is generally 400 IU/kg IM or SQ or 10,000 IU/m^2 IM or SQ. It is available in vials containing 10,000 IU for parenteral administration.

Bleomycin

Bleomycin is a mixture of glycopeptides in which bleomycin A2 is the predominant species in clinical formulations. Bleomycin is a useful drug in veterinary oncology because of the activity against squamous cell carcinomas and the virtual lack of bone marrow toxicity, making it valuable in multidrug regimens. Bleomycin binds to heavy metals (mainly copper and iron) and the bleomycin–iron complex appears responsible for cytotoxicity through direct damage to DNA by inducing single- and double-strand breaks (Lazo and Chabner, 2001). Cellular uptake of bleomycin is slow and there have not been any transporters associated with active transport; thus, large concentration gradients are maintained between the extracellular and intracellular spaces. The uptake of bleomycin can be enhanced with disruption of the cell membrane such as occurs with electrical permeabilization, and the use of electrochemotherapy with bleomycin has been evaluated and reported to be efficacious in veterinary medicine (Spugnini et al., 2006, 2007; Cemazar et al., 2008). Dose-limiting toxicity with bleomycin is generally pulmonary fibrosis, which appears to be cumulative, and a more common but less significant skin toxicity. The unique locations for bleomycin toxicity appear to be caused by the relative lack of the metabolizing enzyme, bleomycin hydrolase, in pulmonary and skin tissues, resulting in higher drug concentrations in these locations. The pulmonary toxicity is enhanced by increased oxygenation, such as occurs during anesthesia, or in locations of prior chest irradiation (Lazo and Chabner, 2001). Toxic skin effects include desquamation, hyperpigmentation, and pruritic erythema. Bleomycin should be used cautiously in patients with pulmonary disease or renal disease.

Bleomycin is not orally bioavailable and can be administered by the intravenous, subcutaneous, intrapleural, or intramuscular route. In electrochemotherapy protocols it is often injected locally to the tumor or tumor bed. Bleomycin is available as a lyophilized powder in 15-Unit vials for reconstitution and parenteral administration.

Cyclooxygenase Inhibitors

Cyclooxygense-2 (COX-2) has been implicated in human carcinogenesis and clear links between inflammation, COX-2 expression, and development of colon cancer have been identified. These drugs are fully discussed in Chapter 20. In addition, use of nonsteroidal antiinflammatory drugs has demonstrated a clear ability to reduce incidence of tumor development in humans. Similarly, there has been recent effort to utilize COX-2 inhibitors in the treatment of cancers based upon the high degree of expression found in many cancer types and a correlation with higher grade or more aggressive phenotype. In veterinary medicine, COX-2 expression has been identified in numerous tumor types as well, including transitional cell carcinoma of the bladder, prostate tumors, osteosarcoma, squamous cell carcinoma, and ovarian carcinomas (Sorenmo et al., 2004; Borzacchiello et al., 2007; Dore, 2011). Piroxicam (Feldene) has received the most attention in cancer treatment as part of combination protocols involving cytotoxic agents but others, such as carprofen, have been evaluated. The mechanism of action is not fully understood but may involve the inhibition of tumor angiogenesis or direct activation of apoptosis in tumor cells (Wolfesberger et al., 2006). Piroxicam therapy has been extensively evaluated in the treatment of canine transitional cell carcinoma (TCC) in combination with other cytotoxic agents (Mohammed et al., 2006; Greene et al., 2007; Robat et al., 2013). COX-2 inhibition with piroxicam combined with metronomic cyclophosphamide has also been shown to have efficacy in incompletely resected soft tissue sarcomas of dogs (Elmslie et al., 2008). Adverse effects of piroxicam include gastrointestinal ulceration and renal papillary necrosis, effects seen with many of the nonsteroidal antiinflammatory drugs. A complete review of nonsteroidal antiinflammatory mechanisms of action, pharmacokinetics, and toxicity can be found in other chapters.

Corticosteroids

Several of the corticosteroid drugs are used in cancer chemotherapy including prednisolone and dexamethasone. Chapter 29 discusses these drugs in detail. The antiinflammatory effects of corticosteroids can help to alleviate pain in patients with terminal disease, and reduction in brain edema secondary to primary and metastatic tumors occurs, particularly with dexamethasone. In addition, steroids may have a direct cytotoxic effect on neoplastic lymphocytes, which appears to be mediated through the glucocorticoid receptor and results in cellular apoptosis (Baxter et al., 1971; Greenstein et al., 2002). When used as a single agent, however, resistance develops rapidly and may be due to populations of cells lacking the glucocorticoid receptor.

Prednisolone is generally administered in combination protocols for treatment of lymphoma, mast cell tumors, and brain tumors and is well-tolerated in dogs and cats. Dog dosing usually starts at 2 mg/kg or 40 mg/m^2 PO daily with tapering of the dose over 3 to 4 weeks. Cats are generally tolerant to longer-term therapy and may be maintained at 5 mg PO every 24 to 48 hours.

Corticosteroids may also be utilized in the management of some of the side effects of other drugs such as hypersensitivity reactions, radiation recall, or sterile hemorrhagic cystitis. A more complete review of the pharmacokinetics and side effects, particularly with regard to suppression of the hypothalamic–pituitary axis, is provided in Chapter 29.

Tyrosine Kinase Inhibitors

In normal cells, regulation of differentiation, growth, survival, and death is accomplished through signals that originate at the membrane and are transmitted through the cytoplasm to the nucleus. Over the past few years, research into the dysregulation of cell-signaling pathways in cancer has led to a greater understanding of how these pathways lead to uncontrolled cell growth and tumorigenesis. Because many of the alterations in these signaling pathways are similar among various tumor types, they have become promising targets for therapeutic intervention. Among the most well studied components of signaling pathways are the receptor tyrosine kinase (RTK) proteins. These membrane spanning proteins contain an extracellular domain, membrane spanning domain, and an intracellular kinase domain, which functions to phosphorylate key tyrosine residues on themselves and other proteins. Upon binding of a growth factor to the extracellular domain, the receptors form a dimer or oligomer, which brings the kinase domains together and allows for autophosphorylation and activation. This activation is then propagated through “second messengers” in the cell, ultimately activating transcription factors that control expression of genes involved in cell differentiation, proliferation, and survival. Oncogenic alterations in RTKs that result in a constitutively activated protein, even in the absence of the growth factor ligand, and result in uncontrolled signaling, are known to contribute to several human cancers and recent research has begun to characterize similar abnormalities in canine and feline cancers. The two most common approaches to inhibition of RTKs in cancer therapy are the development of monoclonal antibodies (MAbs) and small molecule inhibitors, which competitively inhibit ATP binding and prevent the transfer of the phosphate group (phosphorylation) to tyrosine residues. There are currently two small molecule inhibitors of RTKs which have been evaluated for use in veterinary medicine.

Toceranib

Toceranib (Palladia) (Figure 44.22) is a small molecule inhibitor of the split kinase family of RTKs that includes vascular endothelial growth factor receptor (VEGFR), platelet-derived growth factor receptor (PDGFR), mast/stem cell growth factor receptor (c-KIT), Fms-like tyrosine kinase 3 (FLT3), and colony-stimulating factor 1 receptor (CSF1R). Toceranib has demonstrated activity against mast cell tumors (MCT) and is labeled for use in this tumor type. Activity has also been demonstrated for some sarcomas and carcinomas. In the setting of metastatic or recurrent intermediate or high-grade mast cell tumor, toceranib has an overall biological response rate of approximately 60% (this includes complete responders, partial responders, and those with stable disease) and, interestingly, tumors with documented activating mutation in the c-KIT receptor were twice as likely to respond to therapy as those without mutation (London et al., 2009). The combination of toceranib and vinblastine (a cytotoxic commonly used in MCT) has also been evaluated and shown to be effective,

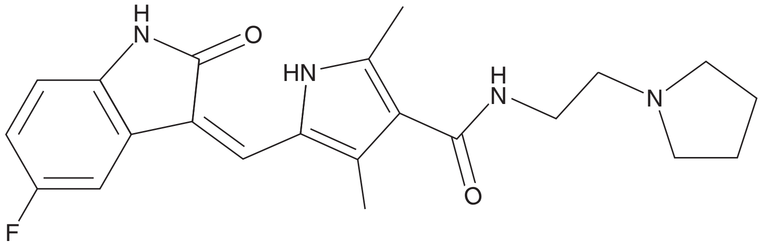


Figure 44.22 Structure of toceranib (Palladia), the first signal transduction inhibitor approved for use in veterinary medicine. Toceranib demonstrates both direct cytotoxic activity through inhibit inhibition of the cKIT oncoprotein on tumor cells as well as antiangiogenic activity through inhibition of vascular endothelial growth factor receptor (VEGFR) and platelet derived growth factor receptor (PDGFR) on tumor vasculature.

although dose reductions in vinblastine were required (Robat et al., 2012). In addition, toceranib has been evaluated in a number of other tumor types and demonstrated a clinical benefit (i.e., complete response, partial response, or stable disease) in anal sac adenocarcinoma, metastatic osteosarcoma, thyroid carcinoma, head and neck carcinoma, and nasal carcinoma (London et al., 2012). Current studies are ongoing and directed at better defining patient populations and tumor types that would benefit from toceranib therapy.

Pharmacokinetic studies of toceranib in dogs have been published and suggest a moderate clearance (1.5 l/kg/h), a large volume of distribution (29 l/kg) suggesting extensive tissue distribution, and a moderate half-life (30 hours) that supports every-other-day dosing (Yancey et al., 2010a). Oral bioavailability is approximately 75% and the majority of the drug and the N-oxide metabolite are excreted in bile with only 7% eliminated in urine (Yancey et al., 2010b). Protein binding is approximately 90% and is independent of plasma drug concentration (Yancey et al., 2010b).

Palladia is metabolized to the N-oxide metabolite through the activity of cytochrome P450 enzymes and thus drug–drug interactions should be considered prior to coadministration of inhibitors or inducer of P450 enzyme activity with regard to increased or decreased exposure to toceranib.

The main toxicities associated with Palladia treatment are gastrointestinal (vomiting/diarrhea) with only mild to moderate neutropenia in most patients. Muscle pain/lameness and protein losing nephropathy have also been reported in dogs receiving toceranib and appear to be cumulative effects. The labeled dose of toceranib is 3.25 mg/kg by mouth every other day and the drug is available as 10, 15, and 50-mg tablets for oral administration.

Masitinib

Masitinib (Kinavet-CA1) is another small molecule inhibitor of c-KIT, PDGFR, and fibroblast growth factor receptor 3 (FGFR3). This drug is also labeled for the treatment of cutaneous mast cell tumors in dogs and has demonstrated a longer time to progression (TTP) and long-term disease control compared to placebo, with a greater effect in tumors that have an activating mutation in the c-KIT receptor (Hahn et al., 2008; Hahn et al., 2010). Toxicities reported with masitinib treatment include gastrointestinal (vomiting/diarrhea), renal (azotemia and proteinuria), hepatic (elevated liver enzymes), and mild to moderate neutropenia. Masitinib is also metabolized by hepatic P450 enzymes and drugs that inhibit P450 activity may increase exposure to masitinib. The initial dose of masitinib is 12.5 mg/kg by mouth, daily with food. Importantly, at the time of publication, masitinib, which was previously conditionally approved, no longer has approval and currently has no legal marketing status. However, this may change and masitinib may become available again to veterinary clinicians in the future.

New Agents

Rabacfosadine

Rabacfosadine (Tanovea CA-1) is a novel, double prodrug of an acyclic nucleotide analog, 9-(2-phosphonylmethoxyethyl) guanine (PMEG). PMEG acts as a competitive inhibitor of DNA polymerases α, δ, and ε, as well as functioning as a substrate for these enzymes, becoming incorporated into DNA, which results in chain termination (Kramata et al., 1998). This leads to S-phase cell cycle arrest and apoptosis. Rabacfosadine selectively accumulates in lymphoid cells where it is converted intracellularly through enzymatic hydrolysis, deamination, and subsequent rapid formation via phosphorylation of the active diphosphate form of PMEG, PMEGpp (Reiser et al., 2008; Vail et al., 2009; Morges et al., 2014). Rabacfosadine is labeled for the treatment of lymphoma in dogs. Activity has been demonstrated in cutaneous T-cell lymphoma (Morges et al., 2014), multicentric lymphoma (Reiser et al., 2008; Vail et al., 2009; Thamm et al., 2017) as well as multiple myeloma (Thamm et al., 2014). A study evaluating the efficacy of rabacfosadine in combination with doxorubicin for treatment-naïve canine lymphoma patients demonstrated that alternating rabacfosadine with doxorubicin provided comparable responses to other standard doxorubicin-based multidrug protocols but required less frequent hospital visits (Thamm et al., 2017). The most commonly reported adverse events following rabacfosadine administration are transient and self-limiting gastrointestinal and hematological toxicity, and 25% or more of dogs may develop dermatopathy, presenting with a wide range of symptoms such as alopecia, otitis externa, ulceration, pyoderma, or excoriations (Morges et al., 2014; Thamm et al., 2014, 2017). Uncommonly, life-threatening or fatal pulmonary fibrosis has been reported as an adverse event and monitoring for this condition is suggested. Additionally, clinical studies of rabacfosadine have often excluded the West Highland white terrier breed because of their predisposition to development of idiopathic pulmonary fibrosis (Heikkila et al., 2011; Heikkila-Laurila and Rajamaki, 2014).

Pharmacokinetic analysis of rabacfosadine in dogs reveals a rapid plasma clearance of parent drug with a half-life of approximately 30 minutes. The cytotoxic PMEG is undetectable in plasma but high concentrations are present in lymphoid cells, while concentrations of the active PMEGpp are nearly 1.5 μM with an intracellular half-life of over 65 hours (Reiser et al., 2008;

Vail et al., 2009). Rabacfosadine (Tanovea-CA1) is available as a 16.4-mg vial for reconstitution and dilution in 0.9% saline and is administered initially at 1 mg/kg as a 30-minute intravenous infusion. Rabacfosadine currently has conditional approval and, as such, may only be used according to the label for treatment of lymphoma in dogs.

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45

Immunosuppressive Drugs

Mark G. Papich

Conditions that require immunosuppressive drugs include skin diseases such as pemphigus, pemphigoid, and lupus-like syndromes. Systemic immune-mediated diseases requiring immunosuppressive drug therapy include immune-mediated hemolytic anemia (IMHA), systemic lupus (SLE), and immune-mediated thrombocytopenia (ITP). For management of some of these disorders, corticosteroids, alkylating agents, cytotoxic drugs, cyclosporine, and gold compounds have been used. Nonsteroidal antiinflammatory drugs (NSAIDs) alone are not usually effective. One of the problems with assessing these medications in veterinary medicine is that there is not enough good quality data to compare drugs, or to understand which one is best for specific conditions. For example, in a systematic review of treatment for IMHA – one of the most important immune-mediated diseases seen by veterinarians – the authors concluded that "This review shows that there are few pieces of high-quality evidence available to inform clinicians managing cases of IMHA in dogs." (Swann and Skelly, 2013).

Glucocorticoids

Glucocorticoids are usually the initial drugs used for treating immune-mediated diseases. They are consistently effective, but have multiple side effects and adverse effects, especially with long-term use. The pharmacology of glucocorticoids is discussed in detail in Chapter 29 and readers are referred to that chapter. Briefly, glucocorticoids exert their action via binding to intracellular receptors, translocating to the nucleus, and binding to receptor sites on responsive genes (Rhen and Cidlowski, 2005; Boumpas et al., 1993), where they modulate the transcription of glucocorticoid-responsive genes (Barnes and Karin, 1997; Barnes, 2001; Hayashi et al., 2004; Rhen and Cidlowski, 2005). By regulating glucocorticoid-responsive genes, protein synthesis is altered, which affects cell function.

Cellular Effects

The mechanism of action for the immunosuppression caused by corticosteroids is complex. In the study by Ammersbach et al. (2006), the authors demonstrated decreased neutrophil migration and survival, decreased cytokines, decreased adhesion expression, and a decrease in the cytotoxic lymphocyte function as well as lymphocyte apoptosis. The dose used in this study in dogs was approximately 2 mg/kg per day for 3 days.

Corticosteroids increase the circulating numbers of mature neutrophils. There is a release of cells from the marginal pool of neutrophils and decreased migration and egress into inflammatory sites. This effect is attributed to decreased expression of adhesion molecules, reduced adherence to the vessel endothelium, and reduced diapedesis from the vessels. Subsequently, there is decreased movement of inflammatory cells into tissues in response to chemotactic stimuli.

Glucocorticoids also affect many functions of macrophages. They suppress the function of macrophages and decrease the inflammatory response, generation of cytokines, and the ability of macrophages to process antigens. They also inhibit phagocytosis by macrophages.

Glucocorticoids affect leukocyte traffic more than cellular function. Corticosteroids decrease the numbers of lymphocytes in the peripheral circulation – caused by a redistribution of circulating lymphocytes – depress lymphocyte activation, and decrease participation of lymphocytes in inflammation. T cells are affected more than B cells. Because B cells are more resistant to the immunosuppressive effects of glucocorticoids, there is minimal effect on immunoglobulin synthesis. However, high corticosteroid doses will decrease immunoglobulin levels, probably because of increased catabolism, as well as through the secondary effects to suppress accessory cells and cytokine synthesis. At antiinflammatory doses, glucocorticoids do not decrease an animal's ability to mount a normal immune response (e.g., from vaccinations) (Nara et al., 1979). Other effects of glucocorticoids include inhibited release of inflammatory cytokines

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from leukocytes (e.g., IL-1, TNF-α, prostaglandins) and decreased expression of cytokines from lymphocytes (e.g., IL-2).

Immunosuppressive Effects

It is difficult to separate the antiinflammatory from the immunosuppressive effects of glucocorticoids. For a complete review of these effects the articles by Cohn (1991) and Barnes (2006) should be consulted. For immune-mediated disorders (e.g., autoimmune skin disease, IMHA, and thrombocytopenia, SLE, immune-mediated polyarthritis) glucocorticoids suppress the immune system response to alleviate clinical signs. In a study in which various treatments for IMHA were examined, corticosteroids alone were as effective as combination treatments (for example, with azathioprine, cyclophosphamide, etc.) (Grundy and Barton, 2001).

Choice of Drugs

For long-term therapy, intermediate-acting steroids (prednisone, prednisolone) are used most often because they can be administered on an every-other-day (EOD) basis. However, injections of dexamethasone are often used for acute, short-term treatment. There are no demonstrated differences in response between dexamethasone and prednisone/prednisolone for acute treatment, but long-term adverse effects may be less for the shorter-acting agents such as prednisone/prednisolone.

Doses

The immune-suppressing effects require higher dosages of corticosteroids than are necessary for antiinflammatory therapy (often by a factor of at least 2-fold), although dose-response relationships have not been fully studied in veterinary medicine. In one study in which the authors documented response to treatment of IMHA, the starting dose of prednisolone was 2 mg/kg per day, followed by a tapering schedule (Piek et al., 2008). In a systematic review of treatment for IMHA, the authors found that glucocorticoids alone will result in a successful outcome in a high proportion of cases, although there is no consensus on the drug dose or duration of treatment (Swann and Skelly, 2013).

Initial (induction) dosage regimens employ daily doses of 2.2 to 6.6 mg/kg (prednisolone or prednisone). Doses greater than 6 mg/kg are rarely used in dogs. Initial doses generally are in the range of 2–4 mg/kg/day. One study reported that the optimal induction doses for skin diseases were 4.4 mg/kg/day for dogs and 6.6 mg/kg/day for cats. A systematic review of treatment outcomes from IMHA in dogs concluded that prednisolone greater than 2 mg/kg every 12 hours are likely to result in unacceptable adverse effects without apparent improvement in short- or long-term outcomes. After an induction period, if the patient responds favorably, the dose may be decreased by approximately 50%. Eventually, one should attempt to maintain the patient on this low dose on an every-other-day EOD basis. Some diseases are slow to respond and a reduction of doses should be performed on a gradual and prolonged schedule. For example, lowering the dose based on an evaluation every 2–4 weeks may be necessary. Eventually, for immune-mediated disease, maintenance doses of 1 mg/kg, EOD are possible but higher doses or adjunctive therapy may be necessary in individual patients.

Adverse Effects

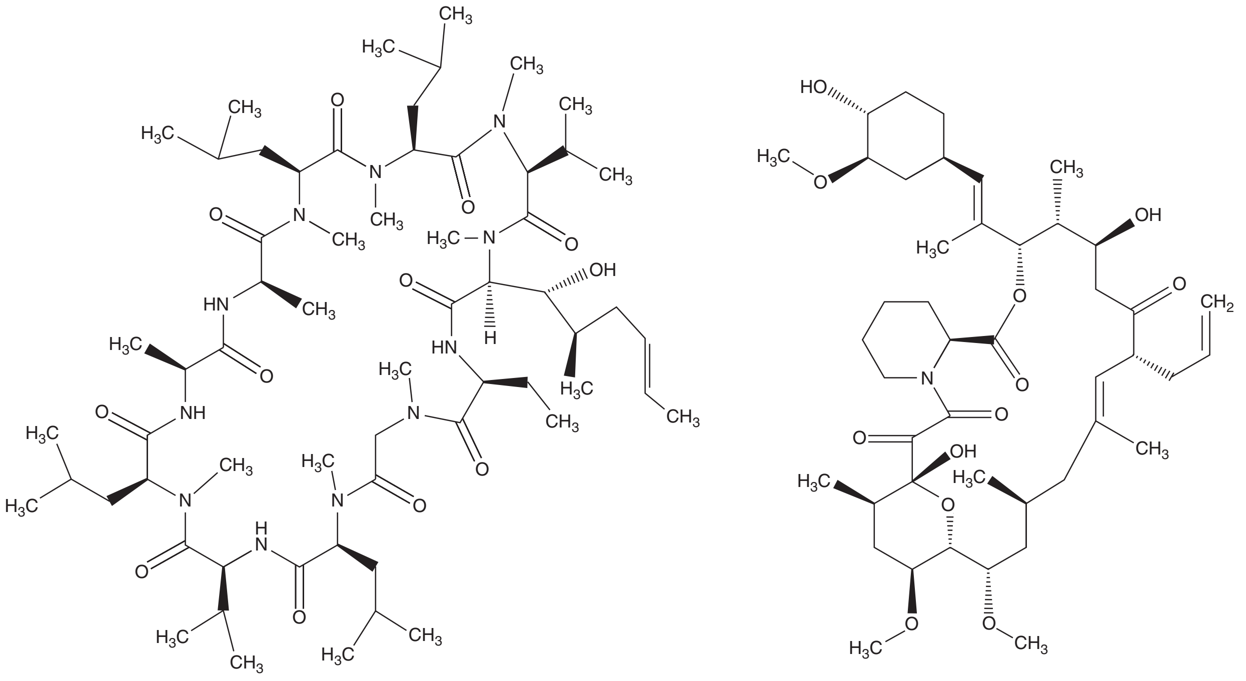
Side effects associated with treatment are discussed in Chapter 29. These effects are not necessarily reasons for discontinuing therapy, and include polyuria/polydipsia, increased appetite (polyphagia), and behavior changes (increased restlessness for example). Adverse effects from immunosuppressive doses of corticosteroids include risk of infectious diseases, adrenal suppression, pancreatitis, delay of healing, catabolic effects on muscle and connective tissues, and hepatopathy.

Rationale for Every-Other-Day Therapy

If a glucocorticoid is used that has a duration of action of 12–36 hours (intermediate-acting), the hypothalamic–pituitary–adrenal (HPA) axis has an opportunity to recover before the next dose. It is important that one chooses a glucocorticoid that does not have a long duration of action for EOD therapy – for example, dexamethasone is unacceptable because its duration is at least 36–48 hours. After 1 mg/kg every 48 hours of prednisolone, adrenocorticotropic hormone (ACTH) was suppressed in dogs for 18 to 24 hours, and returned to normal until the next scheduled dose (Brockus et al., 1999). EOD therapy will minimize, but *will not prevent* adrenal atrophy, and other adverse effects such as those on the immune system and the effects on metabolism.

Cyclosporine

Cyclosporine is a fat-soluble, cyclic polypeptide with immunosuppressive activity (Figure 45.1). It was derived from the soil fungus *Tolypocladium inflatum*. The proper spelling of the name has been a source of confusion. The US adopted name (USAN) is *cyclosporine*. The British Approved Name (BAN) is *ciclosporin*, which is the same as the International Nonproprietary Name (INN). Previously used names are *cyclosporin* and *cyclosporin A*.



Cyclosporine

Tacrolimus

Figure 45.1 Structure of cyclosporine (left) and tacrolimus (right).

The designation of "A" after the name is no longer necessary. It has been an important drug used in humans, primarily to produce immunosuppression in organ transplant patients. It has gained recognition for veterinary use because of availability of a veterinary formulation of cyclosporine (Atopica). The veterinary product is FDA-approved for both dogs and cats. A review published in 2014 (Archer et al., 2014) provides details of previous studies, pharmacokinetics, and studies in dogs.

Cyclosporine binds to a specific cellular receptor on calcineurin and inhibits the T-cell receptor-activated signal transduction pathway by suppressing the nuclear factor of activated T cells (NFAT) (Figure 45.2). Particularly important are its effects to suppress interleukin-2 (IL-2) tumor necrosis factor-α (TNF-α) and other cytokines, and block proliferation of activated T lymphocytes. The action of cyclosporine is more specific for T cells as compared to B cells. It has little effect on humoral immunity. One important advantage in comparison to other immunosuppressive drugs – especially corticosteroids – is that it does not cause significant myelosuppression or suppress nonspecific immunity.

Clinical Use in Dogs

Cyclosporine has been used for a number of diseases in veterinary medicine and since the introduction into veterinary medicine over 10 years ago, the number of potential uses, particularly in dogs and cats, has increased substantially. Many of these diseases have been dermatological, as reviewed by Robson and Burton (2003). In dogs, when used in the treatment of perianal fistulas, (Mathews and Sukhiani, 1997; Griffiths et al., 1999) an 85% healing rate was found in one study (Mathews et al., 1997) (2.5–6 mg/kg/day); in sebaceous adenitis, good response was reported in one case (Carothers et al., 1991); and in idiopathic sterile nodular panniculitis, excellent results were seen in two reported

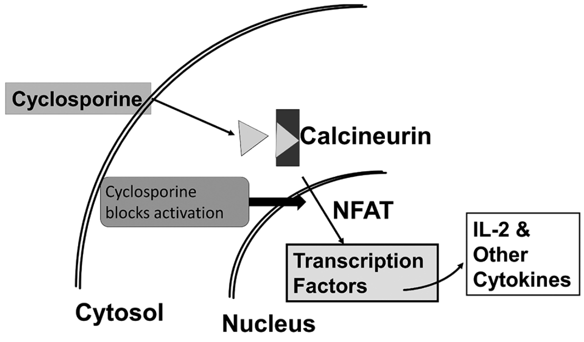


Figure 45.2 Mechanism of action of cyclosporine. Cyclosporine enters the cytosol, where it binds and inhibits calcineurin at a specific receptor site. By inhibiting calcineurin, the movement of the nuclear factor of activated T cells (NFAT) is prevented from entering the nucleus. Without the action of NFAT, transcription factors that produce cytokines is inhibited. (See text for additional details.) Tacrolimus is also capable of inhibiting calcineurin, but at a different binding site.

cases, which were followed for 6 months following discontinuance of the cyclosporine (Guaguère, 2000).

Immune-mediated Diseases

Cyclosporine has been used for treatment of a variety of immune-mediated diseases that include IMHA, inflammatory bowel disease (IBD) (Allenspach et al., 2006), immune-mediated polyarthritis (Rhoades et al., 2016), and aplastic anemia (AA). For some diseases there is only anecdotal accounts of successful outcomes with cyclosporine, and for others (e.g., IMHA) the evidence is of such poor quality that conclusions cannot be made.

For treating immune-mediated skin diseases, the results from pilot studies for treating pemphigus foliaceous in dogs have been disappointing (Rosenkrantz et al., 1989). It did not help any patients with mycosis fungoides. In a study in which five dogs with pemphigus foliaceous were treated, there was little benefit (Olivry et al., 2003a). The dogs with pemphigus foliaceous were initially treated with the “atopy dose” of 5 mg/kg/day. If there was no response the dose was increased to 10 mg/kg/day. At the end of the trial, it was concluded that at 5–10 mg/kg/day cyclosporine was unable to produce complete remission in any of the five dogs. In a case study of immune-mediated polyarthritis in dogs (Clements et al., 2004), dogs were treated with prednisolone and various other immune-modifying drugs. Three dogs treated with cyclosporine at 5 mg/kg per day did not respond.

Atopic Dermatitis

In people it has been used successfully for treatment of atopic dermatitis (Camp et al., 1993). Because of this efficacy, the use of cyclosporine for treating canine atopic dermatitis was initially investigated in a pilot study of 14 dogs (Fontaine and Olivry, 2001), and subsequently in larger controlled studies (Olivry et al., 2002a, 2002b). The evidence for recommending cyclosporine was summarized by a task force (Marsella and Olivry, 2001) and an evidence-based review (Olivry et al., 2003b). In another trial of 30 dogs treated with cyclosporine (5 mg/kg/day) or prednisolone (0.5 mg/kg/day), the efficacy of cyclosporine was not statistically different than prednisolone (Olivry et al., 2002a). Reductions in mean lesion scores were 60% and 59% in prednisolone and cyclosporine groups, respectively. Reduction in mean pruritus scores were 81% and 78% in prednisolone and cyclosporine groups, respectively. In another published study from the same author (Olivry et al., 2002b), 91 dogs with atopic dermatitis were treated with cyclosporine and compared to placebo. Two doses were examined, 2.5 mg/kg/day and 5 mg/kg/day. There was a dose-dependent effect. Dogs treated with 5 mg/kg had statistically significant reduction in pruritus scores (45%) and skin lesions (67%). The dose of 2.5 mg/kg was not statistically different from placebo. Some dogs may benefit from less than once-daily administration, as shown in another study (Steffan et al., 2003). In this study, administration of cyclosporine (5 mg/kg) was compared to methylprednisolone (0.75 mg/kg) for treatment of canine atopic dermatitis. The response was not different between the drugs, but there was an overall better assessment of efficacy and more gastrointestinal problems in dogs treated with cyclosporine. In the cyclosporine-treated dogs, the dose was started at 5 mg/kg/day for 4 weeks, but eventually half of the dogs were adjusted to an EOD dose, and one-quarter of the dogs were given cyclosporine at 5 mg/kg only twice a week.

Clinical Use in Cats

In August 2011, a cyclosporine oral solution (Atopia) was the first drug approved by the FDA for treating allergic dermatitis in cats. Capsules also can be used and one 25-mg capsule has been used for a dose of 25 mg/cat, once daily. The FDA-approved dose is 7 mg/kg per day, which is higher than the canine dose. Higher doses are needed because of the more variable absorption in cats compared to dogs. Other uses for cats include immunosuppression for kidney transplantation (Mathews and Gregory, 1997) at a dose of 3–4 mg/kg q 12 h. It also has been used to treat inflammatory diseases in cats, including stomatitis and eosinophilia granuloma complex (Guaguère and Prèlaud, 2000; Vercelli et al., 2006). For treatment of pemphigus foliaceous in cats it was more effective than in dogs. At a dose of 5 mg/kg per day, it was effective in some cats and steroid sparing (Irwin et al., 2012).

Formulations and Pharmacokinetics

The pharmacokinetics of cyclosporine are complicated because of the differences between dosage forms, presence of many metabolites, influence of drug interactions, and variability in oral absorption. The formulation called *Sandimmune* was used for many years but was absorbed inconsistently and is not used today. The common human formulation is a microemulsion called *Neoral,* which has a much more consistent rate and extent of absorption that is less affected by factors such as feeding than Sandimmune (Lown et al., 1997). There are now over 20 human generic formulations of this formulation. There is no evidence available to show that the human generic formulations are bioequivalent to the brand-name product in dogs and cats. The introduction in late 2003 of Atopica represents the first oral veterinary formulation of cyclosporine. The formulation is exactly the same as Neoral microemulsion (absorption, kinetics, and dissolution), except that there is a greater variety of capsule sizes available (10, 25, 50, and 100 mg) for veterinary use. Compounded formulations can have variable strength and are not recommended for clinical use in dogs and cats.

The pharmacokinetics of cyclosporine in treated animals has been examined (Steffan et al., 2004). Cyclosporine is metabolized in the intestine and liver. Twenty-five to thirty metabolites have been identified. The prehepatic intestinal enzymes account for significant metabolism of cyclosporine (Wu et al., 1995), and systemic absorption in dogs is only 20–30% (Myre et al., 1991). Systemic absorption in cats is similar at 25–29% (Mehl et al., 2003). The intestinal metabolism by cytochrome P450 enzymes (CYP) and the efflux caused by intestinal p-glycoprotein (P-gp) account for most of the loss in systemic availability after oral administration. Drug enzyme inhibitors can inhibit the presystemic metabolism and produce an increase in systemic availability of cyclosporine (see Section Drug Interaction). For example, 5–10 mg/kg of ketoconazole once daily can decrease the dose of cyclosporine because clearance is reduced by 85% (Myre et al., 1991).

Administration

As in the clinical trials cited in this chapter, the effective dose for treating dermatitis in dogs has been 5 mg/kg/day. There may be a delay in response when cyclosporine is initiated for as long as 4–6 weeks and some dermatologists administer corticosteroids for the first 2 weeks when initiating treatment. Some clinicians have been able to lower the dose, or administer the dose every other day, and still maintain clinical remission. Approximately half of treated dogs can be maintained by increasing the dose interval.

Although clinical studies evaluating efficacy for treating immune-mediated diseases in dogs or cats are very limited, cyclosporine has been used for diseases listed in Sections Clinical Use in Dogs and Clinical Use in Cats. For these conditions it is generally assumed that higher doses are needed – generally in the range of 10 mg/kg every 12 hours oral. At these doses, cyclosporine consistently suppressed cytokine expression from activated T cells in dogs for a duration of 12 hours (Fellman et al., 2015). The same authors found that at a lower dose of 5 mg/kg once daily in dogs produced a more variable response and inconsistent suppression of activated T cells (Archer et al., 2011).

Monitoring

For organ transplantation in people, doses of 5–10 mg/kg/day are used to achieve targeted whole blood concentrations of 150–400 ng/ml. In people, dosages are adjusted to meet the needs of the individual patient on the basis of clinical response and monitoring trough plasma concentrations. For organ transplantation in cats (Mathews and Gregory, 1997) a dose of 3 mg/kg q 12 h was used to achieve trough blood concentrations of 300–500 ng/ml. For organ transplant in dogs, 10 mg/kg q 12 h to achieve concentrations of 500–600 ng/ml has been used (Mathews et al., 2000).

A report on treating perianal fistulas in dogs (Mathews and Sukhiani, 1997) found that an average dose of 6 mg/kg q 12 h was needed to achieve a targeted blood concentration of 400–600 ng/ml. However, this recommendation was later modified to 2.5–6 mg/kg/day. The most common dose for treating perianal fistulas is 3 mg/kg q 12 h.

Steffan and colleagues (2004) found that when administering approved doses to dogs with atopic dermatitis, routine monitoring of blood cyclosporine concentrations was not necessary. When animals were administered a dose of 5 mg/kg/day, there did not appear to be a strong correlation between blood concentration achieved and clinical response. It is also difficult to interpret blood concentration monitoring results because no therapeutic range has been established for dogs and cats. Nevertheless, because of wide individual variation in cyclosporine pharmacokinetics, monitoring blood concentrations is sometimes used to determine if the lack of therapeutic response is caused by inadequate oral absorption, rapid metabolism, or poor compliance by the pet owner. Monitoring can sometimes be helpful to identify why patients may be nonresponders. Effective treatment has been reported, even when trough blood concentrations are low. However, according to one paper, clinical benefits have not been observed when trough concentrations have fallen below 50 ng/ml; therefore, blood testing of patients failing therapy was suggested to reveal inadequate dosing (Robson and Burton, 2003).

One must be cognizant of the assay used when monitoring cyclosporine. Plasma values will be lower than whole-blood assays because as much as 50–60% and 10–20% of the dose is concentrated in erythrocytes and leukocytes, respectively. Nonspecific immunoassays will report higher values than a more specific (monoclonal or HPLC) assay. Despite the hypothesized higher specificity when using the monoclonal assay, important discrepancies between these assays and the more specific HPLC assay have been reported (Steimer, 1999).

If trough samples are monitored (to detect the lowest point during the dose interval), samples are collected immediately before the next scheduled dose (Figure 45.3). However, trough concentrations can be low because of the short half-life and may not correlate with clinical response. Effective treatment has been reported, even when trough blood concentrations are low. If peak samples are monitored (to detect the maximum concentration) blood sample monitoring is done by collecting a 2-hour sample (C2), which correlates

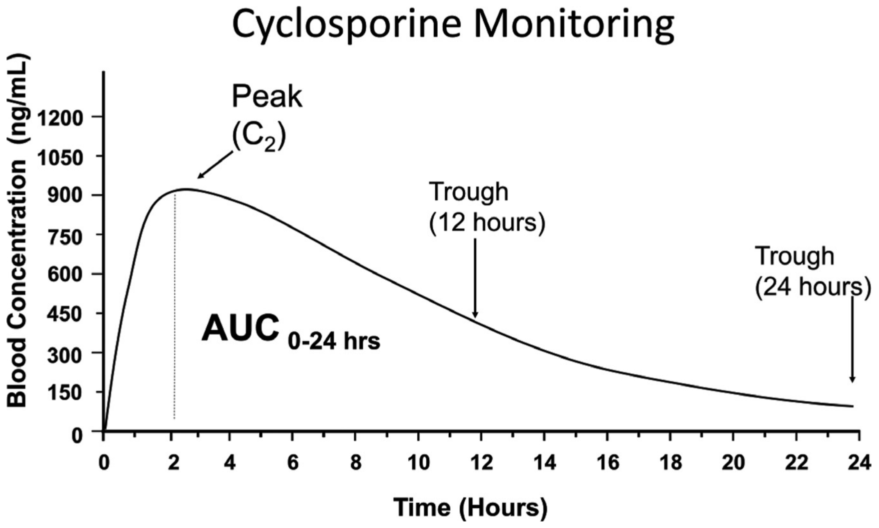


Figure 45.3 Hypothetical blood concentration versus time curve for cyclosporine administration to animals. The peak concentration typically occurs at 2 hours in most animals and can be used to monitor peak effects. This concentration (C_2) is correlated with the total area-under-the-curve (AUC) and is associated with pharmacodynamic effects (see text). If monitoring is performed by collecting a trough sample, there can be differences between the 12 hour sample (with twice-daily dosing) compared to the 24 hour sample (with once-daily dosing).

better with the area-under-the-curve (AUC) than the trough concentration (Figure 45.3). A 2-hour sample collected from dogs was able to detect the peak response, which correlated with immunosuppressive effects better than trough concentrations (Fellman et al., 2015). This confirmation in dogs agrees with evidence in people that a C2 blood sample value correlates better with clinical results than trough samples (Levy et al., 2002; Nashan et al., 2002). If monitoring is performed using a 2-hour sample, anticipate that the drug concentration at 2 hours will be approximately twice as high compared to the levels at 12 hours. The peak concentration to dose ratio in dogs is 179 ± 56. Therefore, at a dose of 5 mg/kg, the average peak concentration is 895 ng/mL with high variability of 31% coefficient of variation (Archer et al., 2014).

Adverse Effects and Precautions

Cyclosporine can cause vomiting, diarrhea, anorexia, secondary infections, and gingival hyperplasia (Table 45.1). Hyperplastic skin lesions have occasionally developed in dogs treated with cyclosporine (Favrot et al., 2005). Papillomavirus can be detected in some skin lesions. Tremors or shaking have been observed in dogs administered high doses. These events are rare and usually not a problem as long as doses are kept within accepted limits.

Table 45.1 Adverse reactions to cyclosporine reported in animals

- Cats: adverse events
  - Anorexia (most common)
  - Vomiting
  - Weight loss, depression
- Dogs: adverse events
  - Vomiting (most common)
  - Diarrhea
  - Depression
  - Lethargy
  - Pruritus
  - Anorexia
  - Increased liver enzymes
  - Gingival hyperplasia

The most common clinical problem with cyclosporine in dogs and cats that has been cited (Olivry et al., 2002a, 2002b, 2003b; Steffan et al., 2003; Heinrich et al., 2011), or detected in the trials conducted by the drug manufacturer (Novartis), is gastrointestinal problems. Vomiting, anorexia, and diarrhea are consistently the most common adverse effect reported, but in some patients may improve several days after initiating treatment. But, in some patients, it has led to discontinue the medication. When anorexia and vomiting are observed, veterinarians have tried various interventions such as lowering the dose, or administration of the dose with some food. Feeding will reduce the amount of cyclosporine absorbed in dogs by 15–20%, but it is unlikely that this decrease will be severe enough to affect efficacy. Keep in mind that some animals may refuse to eat their food if the drug is mixed in with their diet.

Nephrotoxicity, once a problem with older forms, is rare with current formulations. Secondary malignancies because of decreased immune surveillance are a possible complication to long-term therapy but have not been reported in dogs or cats.

Increased risk of toxoplasmosis has been a concern from cyclosporine administered to cats (Lappin et al., 2015). At a dose of 7.5 mg/kg/day (label dose), for an extended (long) interval, cyclosporine increased the severity of *Toxoplasma gondii* infection in naïve (seronegative) cats, but not in cats previously exposed (seropositive) to *Toxoplasma gondii*, and did not activate oocyst shedding in cats that had been previously exposed. Seronegative (naïve) cats may be at risk for developing clinical toxoplasmosis if they are infected while receiving cyclosporine treatment. Other safety concerns in cats were addressed by the manufacturer or in other studies (Roberts et al., 2014). Safety of cyclosporine in cats at high doses for 6 months was assessed, with the conclusion that it is safe and well tolerated.

Glucose metabolism has been studied in dogs (Kovalik et al., 2011). Cyclosporine will negatively affect glucose homeostasis in some animals. The mechanism is via decreased insulin secretion and possibly insulin resistance, but the clinical significance is unclear. Cyclosporine may increase platelet hypercoagulability, which may be a concern when it is used to treat IMHA in dogs (this disease has been associated with thromboembolism). Cyclosporine administration increases thromboxane (TXA_2), which can trigger platelet aggregation and vasoconstriction (Thomason et al., 2012, 2016). Administration of aspirin to dogs will prevent the cyclosporine-induced increase in TXA_2 (Thomason et al., 2016).

Drug Interactions

Several drugs may interact with cyclosporine. These drugs include ketoconazole, itraconazole, fluconazole, erythromycin, and diltiazem. All these drugs can potentially increase concentrations of cyclosporine in animals. For example, coadministration of ketoconazole to treat secondary fungal infections will substantially decrease metabolism of cyclosporine (Myre et al., 1991). Ketoconazole has been used deliberately to reduce the need for cyclosporine in some investigations (Patricelli et al., 2002) and doses have been reduced to one-third of the original dose when administered with ketoconazole. In one study with cyclosporine in the treatment of perianal fistulae, the dose of 1 mg/kg cyclosporine combined with 10 mg/kg ketoconazole was used. The author felt that a dose of 0.5 mg/kg combined with 10 mg/kg ketoconazole also could be used (Mouatt, 2002). In people, grapefruit juice will increase concentrations of cyclosporine because of inhibition of metabolizing enzymes. However, the dose of grapefruit required to produce a similar effect in dogs is very high (41 capsules) and not clinically practical (Hanley et al., 2010). Cyclosporine also is an inhibitor of one of the membrane transport proteins P-glycoprotein. It can inhibit transport of ivermectin and increase risk of ivermectin toxicity in dogs.

Ophthalmic Use.

Keratoconjunctivitis sicca: Cyclosporine (Optimmune) is approved to increase tear production in dogs with keratoconjunctivitis sicca (KCS). Success rates are high. Topical cyclosporine has also been used to treat other immune-mediated diseases of the eye. It is available as a 0.2% ophthalmic ointment.

Anterior uveitis: Cyclosporine intraocular implants have been developed by an ophthalmologist at North Carolina State University (Gilger et al., 2006). These implants are administered to horses to treat anterior uveitis. This method of treatment slowly releases the medication and can produce a long-term sustained therapeutic effect.

Tacrolimus, Pimecrolimus, and Sirolimus (Rapamycin)

Tacrolimus (Prograf) is a macrolide antibiotic produced by *Streptomyces tsukubaensis* (Figure 45.1). It is approved for use in people as 1 and 5-mg capsules and injection. This drug has similar immunological effects on lymphocytes as cyclosporine but is much more potent. Like cyclosporine, it binds to an intracellular receptor that inhibits IL-2 gene expression in T-helper lymphocytes, although the binding site is different than cyclosporine. It also may bind to receptors that inhibit expression of other cytokines. Most experience with tacrolimus has been in experimental studies. It has not been used systemically in veterinary medicine and may produce unacceptable adverse effects in dogs.

Tacrolimus (Protopic) has been used topically in dogs with some dermatoses. Systemic absorption is so low that it is safe for topical administration to dogs. More recently, pimecrolimus has been used in people as a topical treatment for atopic dermatitis. Pimecrolimus (Elidel) is available as a 1% cream. There is minimal systemic absorption of pimecrolimus after application.

Sirolimus, previously known as rapamycin, is similar in structure to tacrolimus, but with different action on the regulatory pathways. It is a macrocyclic lactone produced from the fungus *Streptomyces hygroscopicus*. It has a unique mechanism of action, and actions that are distinct from tacrolimus. Sirolimus inhibits the function of a specific protein kinase known as the mammalian target of rapamycin (mTOR, also called the mechanistic target of rapamycin). This pathway responds to growth factors and is responsible for cell proliferation. The immunosuppressive properties are caused by inhibition of T-lymphocyte activation and proliferation through the IL-2 pathway. It can inhibit cytokine and growth factor pathways and has been used to prevent transplant rejection in people. Although sirolimus has been used for immunosuppression to prevent transplantation graft rejection, more recently it has been explored for cancer protocols.

The limitation for the use of sirolimus in dogs has been toxicity. When administered to experimental dogs undergoing renal transplantation procedures, the dose of 2 mg/kg produced severe toxicosis that included severe gastrointestinal toxicity with mucosa necrosis, submucosa vasculitis, and death. Even at lower doses of 0.5 mg/kg toxicosis was observed. At lower doses of 0.05 mg/kg IV and 0.1 mg/kg IM there was no toxicity, but insufficient immunosuppression. An additional concern is the tissue irritation and abscesses that have been observed in dogs from SC and IM injection. Subsequently, further studies with sirolimus have been conducted at low doses administered by the oral route (Larson et al., 2016). In a pharmacokinetic study of 0.1 mg/kg oral, the half-life was 38 hours after a single dose and 100 hours after multiple doses and the pharmacokinetics were highly variable among dogs (Larson et al., 2016). Clearance also was affected by multiple administration. The concentrations achieved at this dose may be sufficient for cytostatic and antiangiogenic effects in cancer treatment, but may not be sufficient to produce immunosuppression. More work is needed on this agent to determine the optimum safe and effective dose for animals.

Oclacitinib (Apoquel®)

Oclacitinib was approved by the US FDA in May 2013 for control of pruritus in dogs associated with allergic dermatitis and atopic dermatitis (Cosgrove et al., 2013a, 2013b). It is administered as a tablet, at a dose of 0.5 mg/kg (0.4–0.6 mg/kg, with or without food), initially on a twice-daily schedule for the first 14 days, then it is intended to be tapered to a once-daily administration. The tablet size is 3.6, 5.4, or 16 mg.

Mechanism of Action and Activity

Oclacitinib has a unique mechanism of action that acts to inhibit proinflammatory cytokines. It inhibits cytokines involved with pruritus in dogs that are dependent on the Janus kinase (JAK) enzyme (Gonzales et al., 2014). It inhibits preferentially the JAK1 enzyme activity over JAK2 or JAK3, with a 1.8-fold selectivity over JAK2, and 9.9-fold selectivity over JAK3. This preferential activity is important because JAK2 is involved with hematopoiesis and high doses of oclacitinib could suppress hematopoiesis. Of significance for atopic and allergic dermatitis treatment is that oclacitinib inhibits the IL-31 cytokine function and reduces IL-31 induced pruritus in dogs. It also may inhibit the function of other proinflammatory cytokines such as IL-2, IL-4, IL-6, and IL-13 that may be involved with allergy.

Use in dogs: In studies of dogs with atopic dermatitis treated with oclacitinib, the efficacy was 66% and 49% as assessed by pet owners and veterinarians, respectively, and 67% in a study with allergic dermatitis. This was significantly better than placebo and 86% of dogs were withdrawn from the placebo arm of the study. Oclacitinib also was shown to have equal efficacy as prednisolone in dogs, with an onset of efficacy of both treatments in as little as 4 hours (Gadeyne et al., 2014). When atopic dogs were treated with either cyclosporine or oclacitinib, the oclacitinib-treated dogs had a faster onset of action and lower frequency of gastrointestinal problems compared to cyclosporine (Little et al., 2015).

Use in cats: At this time, there is only limited information on use in cats. Some veterinarians have used oclacitinib in cats and reported (anecdotally) a beneficial response. However, oclacitinib is not as potent in cats as in dogs and higher doses may be necessary to achieve the same degree of JAK inhibition. Safety at higher doses has not been established in cats.

Pharmacokinetics

It is rapidly absorbed (89% bioavailability) and reaches a peak in less than 1 hour, producing a quick response after administration (Collard et al., 2014). The rapid onset of action distinguishes it from some of the other products currently used for treatment of atopic dermatitis. After absorption, the half-life is approximately 3–5 hours in dogs (Collard et al., 2014). Pharmacokinetics were not affected by feeding or breed of dog.

Safety and Adverse Effects

During the field study reported to the FDA for approval, there was a low rate of adverse reactions (diarrhea 2.3–4.6%, vomiting, 2.3–3.9%), which were not different from the placebo and usually resolved after initial dosing. In a published study (Cosgrove et al., 2013b) there was a 9.2% incidence of vomiting and 6% incidence of diarrhea in 283 dogs. Treated dogs had decreased leukocytes and serum globulin compared to placebo group. In field trials there have been isolated cases of demodicosis, pyoderma, pneumonia, and mast cell tumor or other neoplasms, but it is not known if these were associated with the medication.

Dermatologists have used oclacitinib with other medications such as corticosteroids, cyclosporine, and other systemic agents but published safety information when administered with these agents is not available. Safety has not been evaluated in breeding animals or during pregnancy.

Cyclophosphamide (Cytoxan®)

Cyclophosphamide is one of the nitrogen mustards and is one of the most potent immunosuppressive drugs available. More details on the pharmacology of cyclophosphamide can be found in Chapter 44.

Mechanism of Action

The immunosuppressive effects of the nitrogen mustards can be attributed to the cytotoxic effect on lymphocytes caused by damage to DNA. Cyclophosphamide is metabolized initially to 4-hydroxyphosphamide and then at target sites metabolized spontaneously (nonenzymatically) to active compounds acrolein and phosphoramide mustard. Phosphoramide mustard is responsible for alkylating bases on the DNA molecule. Because of the cytotoxic action on lymphocytes, it can directly suppress B-cell activity and antibody formation. B cells are affected more than T cells because their rate of recovery from an alkylating agent is slower.

Adverse Effects

Cyclophosphamide is a potent immunosuppressive drug with a high risk of myelosuppression. In dogs the most serious toxic effect is bone marrow suppression, which can lead to secondary infections, thrombocytopenia, and anemia. Another adverse effect is sterile hemorrhagic cystitis. The incidence has been estimated to be as high as 24% in treated dogs and 3.7% in treated cats. The injury to the urinary bladder is caused by the toxic effects of metabolites on the bladder epithelium (especially acrolein) that are concentrated and excreted in the urine. Various strategies have been used to decrease the injury to the bladder epithelium. Corticosteroids are usually administered with cyclophosphamide to induce polyuria and decrease the toxic effect of the drug on the epithelium. In people, and occasionally in dogs, the drug mesna (Mesnex, mercaptoethanesulfonate) has been used to prevent drug-induced cystitis. Mesna provides free active thiol groups to bind metabolites of cyclophosphamide in the urine. Another therapy that has been used to reduce hemorrhagic cystitis is the administration of furosemide. In a study of 216 cases (Charney et al., 2003), there was less hemorrhagic cystitis when furosemide was administered concurrently, compared to cyclophosphamide alone. The dose of furosemide used was 2.2 mg/kg IV administered at the time of cyclophosphamide administration.

Cats are more resistant to hemorrhagic cystitis from cyclophosphamide. Gastrointestinal effects such as nausea, vomiting, and diarrhea also are possible in any species treated.

In people, long-term therapy is discouraged because of the risk that cyclophosphamide will induce secondary malignancies. Secondary malignancies have not been reported in animals that have been treated.

Clinical Use

Compared to other immunosuppressive agents (azathioprine, cyclosporine, mycophenolate, leflunomide), cyclophosphamide has not been used regularly as an immunosuppressive drug in animals. The adverse effects at high doses can be severe, and efficacy has not been documented for diseases in animals (use has been anecdotal). Nevertheless, it is sometimes used as an alternative when other drugs have been ineffective. The dose administered to dogs is 50 mg/m^2, which is approximately 1.5 mg/kg for large dogs and 2.5 mg/kg for small dogs. It is available in 25 and 50-mg tablets. This dose has been administered EOD with corticosteroids administered on the alternate days. Cyclophosphamide also is administered at this dose by some clinicians as a pulse dose on 4 consecutive days/week (4 days on, 3 days off). Cats have received a total dose of 6.25–12.5 mg, once daily, 4 days per week.

For treatment of IMHA, the efficacy of cyclophosphamide has been questioned (Mason et al., 2003). In a study in which dogs were randomized to receive cyclophosphamide plus glucocorticoids or glucocorticoids alone, there was no added benefit from cyclophosphamide. Regeneration of red blood cells was suppressed in cyclophosphamide-treated animals.

Chlorambucil

Chlorambucil (Leukeran) also is a nitrogen mustard and is sometimes used as a substitute for cyclophosphamide. It has a similar action as cyclophosphamide, but is one of the slowest-acting of the class of nitrogen mustards. Although little has been published on the clinical use of chlorambucil, it may be effective in dogs and cats for immune-mediated disease. However, direct comparisons to other immunosuppressive drugs has not been reported. One of the most frequent uses has been for treatment of immune-mediated skin diseases of cats. In one report, cats with pemphigus and eosinophilic granuloma complex (EGC) responded favorably with few side effects (Helton-Rhodes and Shoulberg, 1992). It also has been used for feline pemphigus foliaceous at a dose of 0.17–0.38 mg/kg every other day (Irwin et al., 2012).

Adverse Effects

Chlorambucil lacks some of the adverse effects of cyclophosphamide. This drug has been administered for months to years to people safely. The bone-marrow suppressing effects are only moderate, compared to other nitrogen mustards, and are rapidly reversible. Chlorambucil has not caused cystitis, which is reported for cyclophosphamide.

Clinical Use

The usual dose in cats is 0.1–0.2 mg/kg, orally, q 24 h. The usual dose in dogs is 2–6 mg/m^2 q 24 h. After remission these doses are administered EOD. It is available as 2-mg tablets. The EOD dose may be given with oral prednisolone on alternate days.

Thiopurines (Azathioprine)

As first-line therapy, or as an alternative to nitrogen mustard alkylating agents for treatment of immune-mediated disease in dogs, thiopurines are often administered. The most common of the thiopurines used for this purpose is azathioprine (Imuran).

Mechanism of Action

Despite the widespread use of azathioprine, little is known in dogs about its metabolism, risk of adverse reactions, and mechanism of immune suppression (Rinkardt et al., 1999; Ogilvie et al., 1988). In people, azathioprine is rapidly and spontaneously metabolized to 6-mercaptopurine (6-MP), which is further metabolized to 6-methylmercaptopurine nucleotides (6-MMPN) and 6-thioguanine nucleotides (6-TGNs) (Figure 45.4). Conversion to metabolites may occur at target cells responsible for immune effects because direct administration of 6-MP is not as effective as administration of azathioprine as a prodrug. 6-TGNs are incorporated into DNA and are considered the active cytotoxic metabolites. These metabolites are responsible for the bone-marrow toxicity, cytotoxic effects, and immunosuppression of lymphocytes (Stefan et al., 2004; Lennard et al., 1989). 6-MMPNs, on the other hand, are inactive metabolites, but can produce some adverse effects, such as hepatic injury. An additional metabolite is the methylthioinosine monophosphate (MeTIMP), which has activity to inhibit de novo biosynthesis of purines in vitro, but the clinical significance is not known (Meggitt et al., 2011). Consequence of deficiencies of the enzyme thiopurine methyltransferase (TPMT) on clinical and toxic effects are discussed below.

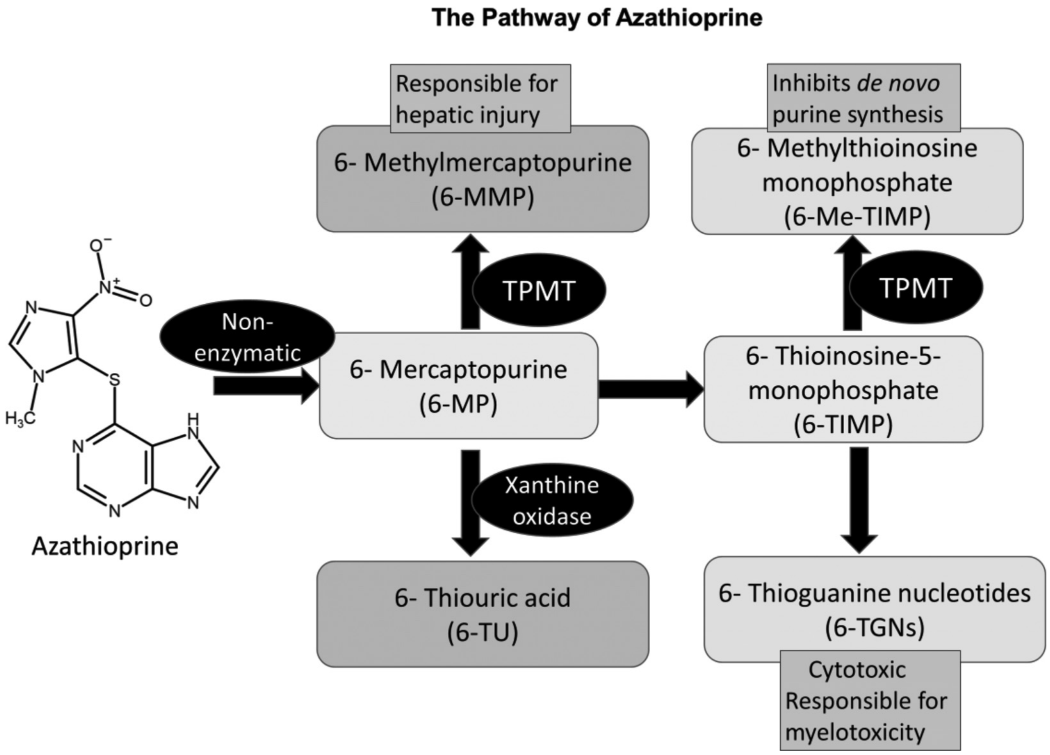

The Pathway of Azathioprine
Azathioprine
Non-enzymatic
6- Mercaptopurine (6-MP)
TPMT
6- Methylmercaptopurine (6-MMP)
Responsible for hepatic injury
Xanthine oxidase
6- Thiouric acid (6-TU)
6- Thioinosine-5-monophosphate (6-TIMP)
TPMT
6- Methylthioinosine monophosphate (6-Me-TIMP)
Inhibits *de novo* purine synthesis
6- Thioguanine nucleotides (6-TGNs)
Cytotoxic Responsible for myelotoxicity

Figure 45.4 Metabolism of azathioprine. Azathioprine is quickly metabolized first to 6-mercaptopurine (6-MP) by a nonenzymatic reaction. 6-MP can then be metabolized to inactive metabolites 6-thiouric acid (6-TU) by xanthine oxidase, 6-methylmercaptopurine (6-MMP) by thiopurine methyltransferase (TPMT), or to active metabolites via 6-thioinosine-5-monophosphate (6-TIMP). Drugs that inhibit xanthine oxidase (e.g., allopurinol) can interfere with this pathway. The 6-thioguanine nucleotides (6-TGNs) can be incorporated into DNA and are thought to be the primary mechanism for the pharmacological effect as well as the myelotoxicity that can be seen in some individuals. The metabolite 6-methylthioinosine monophosphate (6-Me-TIMP) can inhibit the de novo synthesis of purines, but this may not be a significant clinical effect. Deficiencies in the enzyme TPMT in people and cats can lead to myelotoxicity, but this relationship has not been established for dogs.

Clinical Use

In veterinary medicine, azathioprine is one of the most commonly administered drugs to suppress immunity in dogs for treatment of dermatological disease, IMHA, intestinal disease, and other immune-mediated diseases (Grundy and Barton, 2001; Olivry et al., 2004; Hostutler et al., 2004; Weinkle et al., 2005). A systematic review of treatment of IMHA in dogs concluded that when a "second-line" agent was needed in addition to corticosteroids, azathioprine was associated with a more favorable outcome than other agents (Swann and Skelly, 2013). However, there are no good quality prospective studies available to assess the true benefit of adding azathioprine to corticosteroid therapy for these diseases.

Azathioprine is available as 50-mg tablets. It is dosed at 2 mg/kg, orally, q 24 h. Long-term therapy is administered at a dose of 0.5–1.0 mg/kg, every other day, with prednisolone administered on the alternate days. In cats, the doses are much lower, with 0.3 mg/kg being the safest (discussed in Section Problems in Cats). In people, there is a long lag period before successful treatment is recognized with azathioprine, which may be as long as 2–8 months. In veterinary medicine this lag period is probably shorter and therapeutic benefits have been observed after only 3–5 weeks, or less.

Adverse Effects

Azathioprine myelotoxicity has been well documented in dogs (Rinkardt and Kruth, 1996; Rodriguez et al., 2004). Leukopenia and thrombocytopenia can be serious. Animals that are treated with azathioprine should have the CBC monitored during therapy to identify the animals that may be at risk for toxicity and to adjust doses accordingly.

Gastrointestinal effects such as nausea and diarrhea may be only temporary and subside after several days of therapy. In people, elevated levels of 6-MMPNs are associated with hepatotoxicity (Stefan et al., 2004). Canine patients also have developed hepatic toxicity within 2–4 weeks after initiating azathioprine therapy. Signs of hepatopathy include liver enzyme increase and clinical signs that may result in discontinuation of treatment.

Long-term effects related to the immunosuppression produced by azathioprine include *Demodex* infection, recurrent pyoderma, and dermatophyte infections. Sterile hemorrhagic cystitis, the complication cited for cyclophosphamide, has not been seen with azathioprine. There has also been association (but not well documented) between the administration of azathioprine plus prednisolone and the development of acute pancreatitis in dogs. It has been suggested that this is caused by azathioprine's effect on decreasing pancreatic secretion in animals.

Problems in Cats

Some veterinarians have administered azathioprine to cats at a total dose of about 6.25 mg per cat (1/8 tablet) EOD. However, doses of 2.2 mg/kg EOD produced profound neutropenia in cats (Beale et al., 1992). Although doses of 1.1 mg/kg every day, or every other day, for treatment of pemphigus have been administered to cats (Caciolo et al., 1984), other references have discouraged its use in cats because of the bone-marrow suppressing effects, even at this dose (Helton-Rhodes, 1995). Differences in metabolism may explain the susceptibility in cats (discussed in Section Importance of Metabolic Pathway for Determining Toxicity). Because of the concern for toxicity, if azathioprine is used, a recommended dose is 0.3 mg/kg once daily to every other day.

Use in Horses

Based on occasional case reports, azathioprine has been used in horses when other treatments have failed (Divers, 2010). Clinical reports indicate that it is effective for immune-mediate diseases in horses, despite pharmacokinetic data that indicates that it is not well absorbed orally. It has been used for immune-mediated thrombocytopenia, IMHA, polysynovitis, and granulomatous enteritis. The dose used in horses is 3 mg/kg orally, q 24 h.

Importance of Metabolic Pathway for Determining Toxicity

Azathioprine toxicity may have a genetic component. After azathioprine is converted to 6-MP, it is further metabolized to other metabolites (Figure 45.4). One metabolic route is via xanthine oxidase to inactive metabolites. Allopurinol will decrease this route because it inhibits xanthine oxidase. Another metabolic route is via thiopurine methyltransferase (TPMT), which can convert 6-MP to hepatotoxic metabolites. The enzyme TPMT is also responsible for converting 6-thioinosine-5-monophosphate (6-TIMP) to other metabolites. In people there is genetic polymorphism that determines high or low levels of TPMT. People with low TPMT activity may have higher levels of 6-thioguanine nucleotides (6-TGNs) and are more responsive to therapy, but have a high incidence of toxicity (myelosuppression); people with high levels of TPMT activity have low incidence of toxicity but lower efficacy (Lennard et al., 1989). Most of the human population has high TPMT activity, but about 11% have low levels and are more prone to toxicity. In people with low TPMT activity, doses must be lowered.

In dogs, levels of TMPT also are highly variable. TPMT activity varied ninefold in a population of 177 dogs (Kidd et al., 2004). Another study with fewer dogs indicated 90% of dogs showing normal TPMT activity and 10% with low activity (White et al., 1998). The variation appears genetically determined, as it is in people. However, it is not clear whether low levels of TPMT in dogs represent a risk. In some dogs with a history of azathioprine-associated myelotoxicity the animals had either intermediate or high levels of TMPT (Rodriguez et al., 2004).

Cats, as suspected because of their susceptibility to toxicity, have low TPMT activity (Foster et al., 2000). Therefore, cats are sensitive to the toxicity of azathioprine. If azathioprine is used in cats, the dose should be much lower than in dogs.

Mycophenolate

Mycophenolate mofetil is an ester prodrug metabolized to the active compound mycophenolic acid (MPA). T and B lymphocytes are critically dependent on de novo synthesis of purine nucleotides. Mycophenolic acid is a potent inhibitor of inosine monophosphate dehydrogenate (IMPDH), an enzyme important for synthesis of purines in lymphocytes. There are two isoforms of this IMPDH, type I and type II. Type II is the form that is important in activated T- and B-cell lymphocytes and is more sensitive to the effects of mycophenolate. Therefore it suppresses lymphocyte proliferation and decreases antibody synthesis by B cells. It is available as a 250-mg capsule (CellCept). In people it is used as a replacement for azathioprine and has been primarily used for immune suppression in liver and kidney transplant patients. It is usually used in combination with glucocorticoids and/or cyclosporine.

Pharmacokinetics / Pharmacodynamics

At doses of 10, 20, and 30 mg/kg oral, to research dogs that underwent hematopoietic stem cell transplantation, the peak concentration (C_{MAX}) did not increase in proportion to the dose (at 20 mg/kg C_{MAX} was 16 μg/ml), and the authors noted that the doses had no linear association with the plasma mycophenolate concentration (Lange et al., 2008). The half-life was 2.9 hours at the low dose, but 4.6 hours at 30 mg/kg. This is shorter than in people and healthy dogs (Langman et al., 1996), and consequently the area-under-the-curve (AUC) is lower than comparable doses in people. The average half-life in healthy dogs (Langman et al., 1996) has been longer (8–9 hours) but with wide variation. In people, the AUC that corresponds to prevention of graft rejection is 30 μg ml/h, but in the dogs from the transplantation study (Lange et al., 2008) the highest dose (30 mg/kg) produced an AUC of only 21 μg/ml. The concentration needed for 50% inhibition of IMPDH in dogs is 200 μg/ml, but this is higher than in people (Langman et al., 1996). In canine studies, a dose of 20 mg/kg oral does not attain levels high enough to reach this target. Therefore, these doses produce exposures in dogs that may be less than the recommended targets for people.

Clinical Use

Mycophenolate has been used to treat immune-mediated diseases, including IMHA, aplastic anemia, immune-mediated thrombocytopenia, glomerulonephritis, myasthenia gravis, neurological disease, meningoencephalomyelitis, and immune-mediated skin diseases. Experience is mostly limited to some retrospective case reports in the veterinary literature. A common dose is 10–20 mg/kg twice daily, but more research is needed to define the optimum dosage. According to pharmacokinetic studies with mycophenolate in dogs, the short elimination rate may require frequent dosing to achieve successful results in some dogs (Langman et al., 1996). For treatment of pemphigus foliaceous it has been given at a dose of 22–39 mg/kg/day divided into three treatments. It was well-tolerated, but only three out of eight dogs who completed the study were improved (Byrne and Morris, 2001). When administered with corticosteroids for treatment of IMHA (10 mg/kg q 12 h) it was well tolerated, but produced no clinical benefits that were greater than corticosteroids plus other agents (Wang et al., 2013). Myasthenia gravis, which is a T-cell dependent and B-cell mediated process, has been treated with mycophenolate but the results in canine studies have been disappointing. At doses of 10–20 mg/kg there was no significant benefit and the authors concluded that the evidence did not support the use of mycophenolate for treatment of acquired myasthenia gravis in dogs (Dewey et al., 2010). It was reported to produce a partial or complete response in dogs for treatment of meningoencephalomyelitis when combined with corticosteroids, but it had to be discontinued in 20% of the dogs because of adverse gastrointestinal effects (Barnoon et al., 2016). Case reports consisting of small numbers of animals indicated that it may be beneficial for treating IMHA and ITP (West and Hart, 2014; Yau and Bianco, 2014). In a case report, 30 dogs treated with corticosteroids and mycophenolate for IMHA at an average dose of 20 mg/kg per day were compared to other combination protocols (glucocorticoids plus azathioprine, cyclosporine, or immunoglobulin (Wang et al., 2013). There was no difference between the mycophenolate-treated group and the other treatments, except that, at some points of evaluation, the mycophenolate group actually had worse outcomes. The experiences in cats have been limited, but there have been reports of use in cats to treat IMHA.

Adverse Effects

Dogs may be more prone to adverse gastrointestinal effects than other animals. The study cited earlier (Barnoon et al., 2016) reported a 20% incidence of adverse gastrointestinal problems that required discontinuation of treatment. In a case report of five dogs with IMHA treated with mycophenolate at 10–15 mg/kg every 8 hours, all dogs had gastrointestinal toxicity (West and Hart, 2014). The authors concluded that the treatment could not be justified for IMHA at the dose used in their study. The most common effects in dogs were nausea, vomiting, and diarrhea, which is related to a direct effect on the intestinal epithelium. These effects appear to increase at higher doses or more frequent dose intervals.

Leflunomide

Leflunomide (Arava) is an isoxazole immunosuppressive drug. Leflunomide is not active as the parent drug, but is converted to an active metabolite A77 1726 (also known as M1 or teriflunomide) that is responsible for clinical immunosuppressive effects. It inhibits the synthesis of pyrimidine via inhibition of the enzyme dihydro-orotate dehydrogenase. This enzyme is important for the de novo pyrimidine synthesis that is critical for function of activated and stimulated T and B lymphocytes. It also may produce antiinflammatory effects.

After oral absorption, the plasma concentrations of leflunomide are low and insignificant. But there are sufficient concentrations of the metabolite M1 that account for the immunosuppressive action. The metabolite M1 has a very long half-life in people (7–10 days) but is much shorter in dogs (21 hours) (Singer et al., 2011). In cats there was 100% oral absorption and the half-life was longer than in dogs (60 hours at 4 mg/kg oral) but not as long as in people (Mehl et al., 2012). Lymphocyte proliferation in cats at this dose was decreased by approximately 50%. In people, several days are required to accumulate to steady-state levels, and many months are needed for complete elimination after treatment is discontinued. In dogs, steady-state concentrations may be attained earlier, but the more rapid elimination in dogs raises questions about extrapolating dosage regimens from people to dogs. Effective doses for cats have not been established.

Indications and Clinical Uses

Leflunomide is used in people primarily for rheumatoid arthritis. In dogs it has been used for a variety of immune-mediated diseases as a substitute for other drugs such as azathioprine or mycophenolate. These diseases include myasthenia gravis, Evan's syndrome, IMHA, thrombocytopenia, and polymyositis/ polyarthritis (Gregory et al., 1998; Colopy et al., 2010). However, well-controlled clinical studies have not been performed to confirm efficacy and most of the use in veterinary medicine is based on anecdotal reports. Adverse effects in dogs may limit the clinical use (see Section Adverse Effects). Experience in cats is even more limited, except for pharmacokinetic studies and a case report on the use for rheumatoid arthritis (Mehl et al., 2012; Hanna, 2005).

Leflunomide is used most often in dogs for immunosuppressive treatment when other drugs have failed, or when the patient has become refractory to other drugs and a substitute is considered. In dogs it is administered orally, usually at 2 mg/kg every 12 hours, then tapered to 2 mg/kg once daily. For polyarthritis one report indicated that it was "safe and effective" at a dose of 3–4 mg/kg per day (Colopy et al., 2010). In cats, results of pharmacokinetic studies suggest an initial dose of 2 mg/kg for 2 days, followed by 2 mg/kg every other day (Mehl et al., 2012). In both dogs and cats, after an induction period, the dose may be decreased by 25% increments until the patient is stabilized or until the disease resolves.

Adverse Effects

The most common adverse effects in dogs have been decreased appetite and diarrhea. Mild anemia and lethargy also have been observed with clinical use. The most detailed report on adverse effects was in the study by McChesney et al. (1994). They used a canine model for renal transplantation and administered leflunomide to dogs at doses of 2, 4, 8, and 16 mg/kg per day. At the low doses, up to 8 mg/kg, it was safe, but did not produce sufficient immunosuppression and all dogs rejected their grafts. As the dose was increased, the dogs became progressively anorexic. At a dose high enough to produce sufficient immunosuppression (16 mg/kg per day) there was severe toxicity with all dogs dying of anemia, anorexia, and weight loss. The dog may be sensitive to adverse effects because at the high dose of 16 mg/kg, the blood concentrations achieved were >50 μg/ml, which are levels that have been well tolerated in human studies.

Gold Therapy (Chrysotherapy)

Derivatives of gold salts such as aurothioglucose (Solganal) have been used to treat some immune-mediated diseases, but its use in veterinary medicine has diminished considerably. In people, its most common use was for rheumatoid arthritis but newer agents have largely replaced gold therapy. It was originally used in the 1800s to treat tuberculosis. The exact mechanism of action is unknown but it may exert the immunomodulating through its effects on T-lymphocyte cell-mediated immunity or via inhibition of sulfhydral enzyme systems (Kean et al., 1997). There also may be an antiinflammatory effect on macrophages and neutrophils. A disadvantage of gold therapy is that the most effective treatment must be it must be administered IM (usually once per week) and it may take several weeks for positive effects to occur. For example, in people, aurothioglucose has an onset of action of 6–8 weeks; in dogs this may be as long as 6–12 weeks.

Available Drugs

Gold therapy – referred to as chrysotherapy in some textbooks – includes various formulations of gold. Older formulations no longer exist commercially. Previously available formulations included gold sodium thiomalate (Myochrysine) as a 25 or 50 mg/ml injection in sesame oil and aurothioglucose (Solganal) as a 50 mg/ml injection in an aqueous formulation. The only formulation used currently for gold therapy is the oral monomeric gold formulation, auranofin (Ridaura) (Kean al., 1997), which is available in 3-mg capsules.

Clinical Use

Controlled studies are not available in veterinary medicine and case reports of successful therapy in the veterinary literature are lacking. The most common use (although rare) has been treatment of immune-mediated dermatological disease unresponsive to corticosteroids alone, or in cases in which one wishes to decrease the patient's reliance on corticosteroids. More recently, it has gained renewed interest in human medicine for its antiprotozoal properties (Madeira et al., 2012). Oral auranofin appears to concentrate in intestinal cells and may be effective for intestinal diseases caused by protozoa. This use has not been reported in veterinary medicine.

Doses

The injectable dose is usually administered as a 1 mg test dose in small dogs or 5 mg to larger canine patients. Doses have been administered once per week until remission, and usually maintenance doses are in the range of 1–2 mg/kg every 2–4 weeks. Doses for the oral drug auranofin have not been established for dogs or cats.

Adverse Effects

The adverse effects are described in people, in which there is a 10–50% incidence, but they are not well documented in animals. Reported adverse effects include dermatitis (which could be confused with the underlying problem being treated), nephrotoxicity, and blood dyscrasias. When administered to animals it is recommended to monitor CBC periodically to detect drug-induced problems. Diarrhea has been reported with the oral product. In pregnant animals these drugs should not be used. They cause abortion and fetal resorption or congenital malformations in laboratory animals.

Dapsone

Dapsone is a sulfone, which shares chemical properties with sulfonamides. It has been in use for over 50 years in human medicine, but use has declined significantly in favor of other agents. It has been used in combination with other drugs to treat leprosy in humans caused by *Mycobacterium leprae*. Other uses include prophylaxis for malaria and treatment of *Pneumocystis carinii* infections in AIDS patients. The dermatological uses include treatment of dermatitis herpetiformis, SLE, pemphigoid, subcorneal pustular dermatosis, and other inflammatory dermatoses.

Mechanism of Action

For treating leprosy, dapsone acts as a folate antagonist, similar to sulfonamides. For other conditions, the mechanism is unknown, but its action appears to be attributed to a suppression of neutrophil function (Booth et al., 1992). It may suppress respiratory burst in inflammatory cells.

Pharmacokinetics

Similarities in metabolism to the sulfonamides are expected. It is highly absorbed with a long elimination half-life of 11–13 hours in experimental dogs. Comparison of metabolic pathway between dogs and people and between cats and people may explain susceptibility to toxicity (see Section Adverse Effects). In people, one pathway for metabolism is acetylation (N-acetylation). The other metabolite in people is a hydroxylamine metabolite (N-hydroxylation). This hydroxylamine metabolite is responsible for adverse effects caused by dapsone, such as methemoglobinemia (Helton-Rhodes and Shoulberg, 1992). Dogs cannot acetylate drugs and a pattern of metabolism of sulfonamides has been identified in dogs that indicates that this lack of acetylation may account for a greater accumulation of toxic metabolites and increased risk of adverse effects (see discussion of sulfonamides in Chapter 32). The same metabolic fate may explain the risk of adverse effects from dapsone in dogs.

Clinical Use in Animals

In animals, dapsone has been used for feline leprosy, although efficacy for this disease is uncertain. In dogs, it has been used for dermatological conditions characterized by vascular disorders, inflammatory skin disease, immune-mediated disease, and subcorneal pustular dermatosis. Since specific studies have not been performed, doses are extrapolated from humans or based on empiricism. Doses listed in textbooks are in the range of 1 mg/kg once, twice, or three times daily. It is available in 25 and 100-mg generic tablets.

Adverse Effects

The adverse effects in animals are not well documented, but can be severe enough to discontinue therapy. In people, dapsone causes agranulocytosis, aplastic anemia, methemoglobinemia, and blood dyscrasias. Toxic dermatological reactions (e.g., toxic epidermal necrolysis) may occur. Because of the similarity to sulfonamides, do not administer to animals sensitive to sulfonamides. Since some dogs may have an inability to detoxify the hydroxylamine metabolite, this could possibly lead to a higher risk of toxicity. Cats are reported to be susceptible to hepatitis and blood dyscrasias. In people, one of the metabolites responsible for toxicity is excreted as a glucuronide metabolite. Cats may be unable to rapidly glucuronate this metabolite, which may increase a cat's risk of toxicosis compared to other animals.

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46

Drugs for Treating Gastrointestinal Diseases

Mark G. Papich

Antiemetic Drugs

Neurotransmitters Involved in Vomiting

Several neurotransmitters are important for stimulating vomiting (Table 46.1) and successful therapy involves blocking one or more of the receptors for these neurotransmitters. There are differences between dogs and cats with respect to the importance of various receptors, and there are also differences between dogs, cats, and people. Dogs respond readily to administration of apomorphine, which is an agonist for dopamine. Cats vomit more readily from stimulation of α_2-adrenergic receptors than dogs, and respond more consistently to administration of the α_2-adrenergic agonists xylazine or dexmedetomidine. Histamine seems to be a more important neurotransmitter in the chemoreceptor trigger zone (CRTZ) in people than dogs or cats. These differences and overlapping effects of neurotransmitters can be confusing, but some generalities can be made, as follows.

- In the emetic (vomiting), histamine (H_1), acetylcholine (muscarinic M_1), serotonin (5-HT_3), dopamine (DA_2), and neurokinin (NK-1) all play some role, although the importance may vary among species.
- In the CRTZ, dopamine, serotonin, and acetylcholine (muscarinic) all play a role. Histamine has uncertain importance in dogs and cats. Drugs that block dopamine can be effective antiemetics from stimulation of the CRTZ in most animals.
- In the vestibular apparatus (motion sickness) acetylcholine (muscarinic receptor) and histamine (H_1) are important. Serotonin and adrenergic receptors also may play a role. Anticholinergic drugs (e.g., scopolamine) and antihistaminic drugs (e.g., diphenhydramine) can produce an antimotion sickness, antiemetic effect in people, but are less effective in dogs. Some of this may be caused by pharmacokinetic differences.
- Afferents from the gastrointestinal (GI) system may mediate vomiting. Cancer drugs (e.g., cisplatin) injure the GI tract and release serotonin (5-HT_3). Cancer drugs also may stimulate the CRTZ directly.
- Neurokinin (substance P, NK-1) is a more recently described neurotransmitter that stimulates vomiting from several sources. NK-1 receptors are found in the emetic center and the CRTZ, but the most important are those receptors in the emetic center. Drugs described as NK-1 receptor blockers (e.g., maropitant, aprepitant) block vomiting from several sources, but seem to be less effective for alleviating nausea.

Phenothiazine Tranquilizers

Dopamine is one of the neurotransmitters in the emetic center and CRTZ for stimulating vomiting at the DA_2 receptor. Phenothiazine tranquilizers such as chlorpromazine (Thorazine®), prochlorperazine (Compazine®), promethazine, and acepromazine antagonize stimulation from dopamine in the CNS (Table 46.2). In addition to the antidopamine effects, these drugs can block alpha-adrenergic receptors (α_1) in the emetic center. Some phenothiazines also block the histaminic and muscarinic (M_1) receptors. Because of this broad action, and ability to inhibit vomiting from a variety of stimuli, this group of drugs has been called “broad-spectrum” antiemetics. Their side effects may limit clinical use in some patients. These side effects include sedation, altered involuntary motor activity (extrapyramidal signs), and peripheral α-adrenergic receptor blockade (vasodilation). Metoclopramide (discussed in section Metoclopramide (Reglan®)) also has antiemetic effects through its action on the DA_2 receptor.

Antimuscarinic Drugs

Drugs that block muscarinic receptors (muscarinic M_1 receptor) include atropine, scopolamine, aminopentamide (Centrine®), and isopropamide (isopropamide is combined with prochlorperazine in Darbazine®) (Table 46.2). These drugs decrease vomiting from various

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Table 46.1 Neurotransmitters important in vomiting

Dopamine, type 2 (DA_2)
Histamine (H_1)
Serotonin type 3 ($5\text{-}HT_3$)
Acetylcholine, muscarinic type 1 (M_1)
Substance P, neurokinin (NK-1)

Table 46.2 Antiemetic drugs and doses

Antihistamines	
diphenhydramine (Benadryl®)	2–4 mg/kg, IM, PO, q8–12h
dimenhydrinate (Dramamine®)	4–8 mg/kg, PO, q8–12h
promethazine (Phenergan®)	0.2–0.4 mg/kg, PO, IM, q8h
Phenothiazines	
chlorpromazine (Thorazine®)	0.5 mg/kg, IM, PO, q6–8h
prochlorperazine (Compazine®)	0.1–0.5 mg/kg, IM, q6–8h
Acepromazine	0.1 mg/kg, IM, PO, q12h
triflupromazine (Vesprin®)	0.1–0.3 mg/kg, IM, PO, q8–12h
trifluperazine (Stelazine®)	0.03 mg/kg, IM, q12h
triethylperazine (Torecan®)	0.13–0.2 mg/kg, IM, q8–12h
metoclopramide (Reglan®)	0.1–0.3 mg/kg, IM, PO, q12h
Anticholinergic drugs	
Atropine	0.02–0.04 mg/kg, IM, SC, q8–12h
methscopolamine (Pamine®)	0.3–1.0 mg/kg, PO, q8h
isopropamide (Darbid®)	0.2–1.0 mg/kg, PO, q12h
Antiserotonin drugs	
ondansetron (Zofran®)	0.5–1.0 mg/kg, IV or oral 30 min prior to administration of cancer drugs For vomiting from other causes, 0.1–0.2 mg/kg slow IV injection and repeated every 6–12h to control vomiting
NK-1 Receptor blockers	
Aprepitant (Emend®, human drug)	1 mg/kg PO, q24h.
Maropitant (Cerenia®)	1 mg/kg SC, q24h, 2 mg/kg oral q24h, or 8 mg/kg oral (for prevention of motion sickness)

causes, including vomiting caused by vestibular stimulation (motion sickness) and stimulation of the CRTZ. In people, scopolamine has been one of the most effective agents for treating motion sickness (Transderm-Scop®), but it is less effective in dogs and cats. The adverse effects of anticholinergic drugs include xerostomia, decreased stomach emptying, ileus, urine retention, and constipation. These drugs are not practical for many of the causes of vomiting that are encountered in veterinary medicine. The adverse effects, including potential for ileus, can lead to other complications. These agents should be used cautiously in patients with glaucoma because there is a risk of increased intraocular pressure. Scopolamine has produced excitement in animals, particularly cats.

Antihistaminic Drugs

The histamine H_1 and H_2 receptor is involved in transmission of vomiting. Antihistamine drugs are discussed further in Chapter 19. Histaminic nerve transmission stimulates vomiting from the CRTZ and vestibular apparatus. This pathway does not appear to be important for cats, and is less important in dogs than in people. Antihistamines also can produce antimuscarinic (atropine-like) effects as well. Some of the phenothiazine drugs also act on histamine receptors and there is some crossover between these drug groups. Antihistamine drugs administered to control vomiting have included diphenhydramine (Benadryl®), dimenhydrinate (Dramamine®), promethazine (Phenergan®, a phenothiazine with antihistamine effects), and cyclizine, (Marezine®) (Table 46.2). Dimenhydrinate and diphenhydramine contain the same drug. Dimenhydrinate contains 54% diphenhydramine and 46% 8-chlorotheophylline. Theophylline is added to the human formulation to decrease the drowsiness from the antihistamine component. In dogs there was greater oral absorption of diphenhydramine when administered as dimenhydrinate than from diphenhydramine alone.

There are few published data on pharmacokinetics of antihistamine drugs in dogs or cats. Diphenhydramine oral absorption was only 8% in dogs and was highly variable. Oral absorption was better when administered as dimenhydrinate (23%) and had a half-life of 11.6 hours, but was highly variable. The half-life of diphenhydramine injection is only 2 hours in dogs. For other antihistamines, the perceived lack of efficacy of antihistamines that has been reported may be caused by poor oral bioavailability, rather than lack of effect at the receptor.

Adverse Effects

The antihistamines are relatively safe drugs. The most significant side effect of therapy is sedation, but this usually is not undesirable in most patients being treated for vomiting. Sedation occurs because the H_1 receptor regulates sleep–wake cycles. The ethanolamines (diphenhydramine and dimenhydrinate) have the greatest sedative effects. Paradoxically, some of the antihistamines have caused excitement in cats.

Metoclopramide (Reglan®)

Metoclopramide has antiemetic effects via three mechanisms: (i) metoclopramide (at low doses) inhibits dopaminergic (DA_2) transmission in the CNS, similar to phenothiazines; (ii) metoclopramide has peripheral effects on the GI tract that increase emptying of the stomach and upper duodenum; (iii) metoclopramide (at high doses) inhibits serotonin (5-HT_3) receptors. 5-HT_3 stimulates vomiting in dogs and cats either in the CRTZ or vagal afferent neurons. Metoclopramide is a broad-spectrum antiemetic that has been popular in small animal medicine, particularly for animals that vomit because of drug therapy (e.g., cancer drugs) or gastrointestinal disease. However, for control of vomiting caused by cancer drugs the results have often been disappointing (Fukui et al., 1992). This drug is also discussed later in the section on prokinetic agents.

Pharmacokinetics

There is large variability in the pharmacokinetics of metoclopramide in dogs. The half-life in dogs has ranged from about 100 to 190 minutes; the systemic availability of an oral dose is only about 50%. The dose administered to dogs and cats is based in extrapolation and empirical use rather than pharmacokinetic studies.

Adverse Effects

Metoclopramide is a dopamine antagonist and at high dosages it can produce adverse reactions that are similar to those caused by phenothiazine drugs. Adverse CNS effects from metoclopramide include excitement and behavior changes. To produce adequate antiemetic effects from blocking 5-HT receptors, high doses may be needed, which increase the risk of extrapyramidal CNS effects attributed to central dopamine (DA_2) antagonism.

The increase in GI motility may produce some abdominal discomfort. Because metoclopramide stimulates upper GI motility, it should not be administered if there is an obstruction. Metoclopramide produces transient endocrine changes, including an increase in prolactin, but the significance of this effect in dogs and cats has not been determined.

Serotonin Antagonists

The pharmacology of serotonin and the antagonists are covered in more detail in Chapter 19. Specific serotonin antagonists include ondansetron (Zofran), granisetron (Kytril), palonosetron (Aloxi), and dolasetron (Anzemet). New drugs in development include tropisetron and azasetron. Of these, ondansetron (Zofran®) is the best known to veterinarians of the specific serotonin (5-HT_3) inhibitors.

Ondansetron has been used to treat vomiting associated with cancer chemotherapy as well as nausea and vomiting caused by gastroenteritis, pancreatitis, and inflammatory bowel disease. In cats, the oral absorption was 32% from oral administration and 75% from subcutaneous administration. The half-life in cats was 1.8 hours from IV administration, 1.2 hours from oral administration, and more prolonged (3.2 hours) from subcutaneous administration. In dogs, it is much less bioavailable (<10%) after oral administration and shorter half-life of 30 minutes, raising questions about the clinical effectiveness of ondansetron in dogs.

The doses used for ondansetron are 0.5–1.0 mg/kg, IV or oral 30 minutes prior to administration of cancer drugs. For other diseases it has been administered at a dose of 0.1–0.2 mg/kg to dogs and cats and increased to 0.5 mg/kg if necessary, and given as often as every 12 hours. Because of concerns regarding oral absorption in dogs to obtain full effects, it should be administered intravenously. Other serotonin antagonists used for antiemetic therapy include granisetron, ondansetron, dolasetron, azasetron, and tropisetron.

As discussed in Section Metoclopramide (Reglan®), metoclopramide at high doses of 1–3 mg/kg can act as a serotonin antagonist and have been used as an IV infusion during cancer chemotherapy to decrease vomiting; but adverse effects are more likely at these high doses.

Glucocorticoids

Glucocorticoids such as dexamethasone have antiemetic properties, possibly by inhibiting prostaglandin synthesis. These drugs are used sometimes in addition to other drugs for decreasing vomiting, particularly when it is caused by cancer chemotherapy. Glucocorticoids are covered in more detail in Chapter 29.

Cannabinoids

Cannabinoids have been used in people who have not responded to any other antiemetic drugs (e.g., patients that are receiving anticancer drugs). The availability of medical marijuana and prescription forms of cannabinoids have increase the interest in the use in veterinary medicine. They have also been used to increase the appetite in human patients with terminal disease, cancer, and AIDS. Except for anecdotal accounts, the use has not been reported in veterinary patients. These agents have been used occasionally by some veterinarians to increase the appetite in cats, but these effects have been untested in clinical studies.

There are approximately 100 cannabinoids in the cannabis plant, but delta-9 tetrahydrocannabinol (Δ^9-THC) is believed to be the most pharmacologically active. There are two important cannabinoid receptors: CB1 and CB2. The CB2 receptor is almost entirely peripheral and responsible for antiinflammatory and antinociceptive effects. The CB1 receptor is located centrally and is responsible for antinausea, psychoactive effects, appetite stimulation, and other central nervous system properties (Vemuri and Makriyannis, 2015). The effect on nausea and vomiting may occur from binding to the CB1 cannabinoid receptor in the emetic center.

The active ingredient in many cannabinoid products is Δ^9-THC. Δ^{9-}THC is a partial agonist for CB1 and CB2. Synthetic marijuana (THC) is available as a prescription drug (dronabinol) for antiemetic therapy; it is marketed as Marinol®. Cannabinoids are relatively well tolerated in people, but side effects include drowsiness, dizziness, ataxia, and disorientation. Withdrawal signs may occur after abrupt discontinuation following repeated doses. For dronabinol, the oral absorption is good, but bioavailability is low due to high first-pass effects. The volume of distribution is very high.

Suggested doses for dronabinol (Marinol®) in animals (but untested in clinical trials) are 5 mg/m^2, and increased as needed up to 15 mg/m^2 for antiemetic administration prior to chemotherapy. For appetite stimulation in animals, doses start at 2.5 mg before meals. It is available as 2.5, 5, and 10-mg capsules.

NK-1 Receptor Antagonists

The neurotransmitter neurokinin-1 (NK-1, also known as substance P) has several functions that include regulating vascular tone and permeability, mucus production and bronchial tone in the respiratory tract, heart rate, and some activity in the central nervous system. The effects on the emetic center led to the development of a new class of antiemetics to antagonize this receptor. The first one approved for use in people for cancer chemotherapy-induced vomiting was aprepitant (Emend®), which was the first substance P/neurokinin-1 (NK-1) receptor antagonist (Dando and Perry, 2004). The use of NK-1 receptor antagonists such as aprepitant in dogs was primarily experimental until the approval of a specific veterinary drug, maropitant citrate (Cerenia®) (Wu et al., 2004; Watson et al., 1995; Huskey et al., 2004; Fukuda et al., 1999; de la Puente-Redondo et al., 2007a; Vail et al., 2007). This agent was approved for use in dogs in 2007, and later approved for cats in 2012. It is available as a 10 mg/ml injection to be administered 1 mg/kg once daily SC for up to 5 days, or a tablet 16, 24, 60, or 160 mg to be administered at a dose of 2 mg/kg per day, PO, for up to 5 days. The tablet can also be used to prevent vomiting caused by motion sickness (vestibulitis) at a dose of 8 mg/kg for up to 2 days. The dosing interval can also be extended to 14 days without significant accumulation as described below in Section Pharmacokinetic. The original approval for dogs and cats restricted injection of maropitant to the SC route. However, some patients may have a painful reaction to SC injection (discussed in Section Adverse Effects). Subsequently, the sponsor obtained FDA approval for intravenous route of administration in dogs and cats to prevent vomiting associated with anesthesia and medical procedures.

The efficacy of maropitant is attributed to its central site of action. It blocks neurokinin-1 (NK-1) receptors in the area of the brain called the *nucleus tractus solitarius* – the site known as the *emetic center*. Input into the emetic center may come from different sources: gastrointestinal tract, higher brain centers, CRTZ, and vestibular apparatus (motion sickness). These inputs also utilize a variety of neurotransmitters: dopamine, histamine, acetylcholine, and serotonin. Traditionally, to block emesis from these stimuli, multiple drugs may be needed: antihistamines, antidopamine drugs (e.g., metoclopramide), dopamine antagonists (e.g., phenothiazines), and serotonin antagonists (e.g., ondansetron). Because NK-1 inhibitors – such as maropitant – block input from multiple sources regardless of the input *they are considered broad spectrum* in action.

Pharmacokinetics

Maropitant is extensively metabolized by the liver and there is little clearance by the kidney; therefore, no dose adjustments are needed in patients with compromised kidney function. There are primarily two canine isoforms involved with metabolism: CYP2D15 and CYP3A1 (Benchaoui et al., 2007a). Absorption from SC administration is rapid and complete (91% absorption), but oral absorption is limited by the first-pass metabolic effects (24% at 2 mg/kg and 37% at 8 mg/kg) (Benchaoui et al., 2007a). The half-life is 7.75 hours and 4–5.5 hours after SC and oral administration, respectively. However, the effects persist for at least 24 hours after a dose, presumably because of binding to the CNS NK-1 receptor that maintains the drug at the site of action. Penetration across the blood–brain barrier has been demonstrated (de la Puente-Redondo, 2007c), which is necessary for activity in the emetic center.

After repeated administration for 14 days, the half-life was 9.22 hours (mean) and 22 hours after doses of 2 mg/kg and 8 mg/kg, respectively (Lesman et al., 2013). In early label warnings there was a concern about accumulation of maropitant after administration consecutively for more than 5 days. However, follow-up pharmacokinetic studies examined administration of maropitant to dogs at a dose of 2 and 8 mg/kg for 14 consecutive days and steady state was achieved after four doses for once-daily 2 mg/kg and after eight doses for daily 8 mg/kg administration. The explanation for this lack of significant accumulation caused by saturation is that even though saturation of the CYP2D15 occurs at the label dosages causing nonlinear pharmacokinetics, at higher concentrations, the CYP3A12 becomes the primary metabolizing enzyme and linear pharmacokinetics are re-established (Lesman et al., 2013). Therefore, there was not significant accumulation used at the low dose of 2 mg/kg or the high dose of 8 mg/kg for 14 days.

Maropitant in Dogs

Maropitant is an effective antiemetic for a variety of stimuli, including cisplatin (chemotherapy)-induced vomiting, pancreatitis, enteritis, copper sulfate and apomorphine-induced vomiting, motion sickness, and ipecac-induced vomiting. In clinical and research trials, maropitant has been effective to treat and prevent chemotherapy-induced vomiting in dogs (Vail et al., 2007; de la Puente-Redondo, 2007a; Benchaoui et al., 2007b) and a wide range of stimuli such as viral disease, food and toxin ingestion, enteropathy, and other diseases (de la Puente-Redondo et al., 2007b). It was more effective than metoclopramide in this trial.

Maropitant has been effective in dogs that were prone to vomiting because of opiate premedication (Lorenzutti et al., 2016; Kraus, 2013, 2014a; Koh et al., 2014; Claude et al., 2014). In these trials, it inhibited vomiting caused by morphine and hydromorphone. The dose in these studies (1 mg/kg, SC or 2 mg/kg oral) was more effective if administered prior to the anesthesia medication (30–60 minutes for injection or 2 hours for oral). In some of the studies in dogs (Kraus, 2014b; Lorenzutti et al., 2016; Benchaoui et al., 2007a) maropitant was effective for reducing vomiting, but less effective for relieving nausea. This observation may indicate that maropitant is highly effective at the vomiting center to decrease emesis, but less effective for preventing the feelings of nausea.

Maropitant in Cats

Maropitant (Cerenia) was approved by the FDA for use in cats in 2012. The antiemetic effects have been demonstrated in both clinical and research cats (Hickman et al., 2009). Oral absorption is higher than dogs (50%) and the long half-life in cats (13–17 hours) is suitable for once-daily administration. It has been tolerated at doses of 0.5–5 mg/kg SC every day for 15 doses. The recommended dose for cats is 1 mg/kg for vomiting caused by motion sickness, and other causes that stimulate the vomiting center.

In addition to the uses listed above, maropitant was effective for reducing the incidence of vomiting associated with dexmedetomidine and morphine (Martin-Flores et al., 2016). However, as observed in the canine studies described above, it was more effective for reducing vomiting than for reducing nausea. In a study in cats with chronic kidney disease, maropitant prevented vomiting in these cats, but did not improve appetite or weight gain (Quimby et al., 2015). Therefore, as observed in some studies with dogs, maropitant may be effective for preventing vomiting, but may not prevent nausea to a similar degree.

Adverse Effects

Compared to other antiemetic drugs, maropitant has had a good safety profile. There are NK-1, -2, and -3 receptors throughout the body. Maropitant has little binding affinity to NK receptors, other than NK-1; it also does not bind to other CNS receptors such as GABA, opiate, adrenergic, serotonin, histamine, muscarinic, or dopamine receptors. Although NK-1 receptors are involved in a large number of other physiological and behavioral responses, at doses used for antiemesis in dogs, there were no adverse effects associated with these other functions.

One of the adverse effects reported is pain or a stinging response when injected subcutaneously (SC). This is caused by the irritant effect of the drug formulation when it dissociates at room temperature (Narishetty et al., 2009). Maropitant forms a molecular inclusion complex with a clodextrin in the formulation but, at refrigerated temperatures, the drug remains complexed within the cyclodextrin cavity. This complex provides higher drug solubility and improved injection-site tolerance. Entrapment of drugs in this cyclodextrin cavity prevents the direct contact of the drug with biological membranes by limiting the free drug concentration and thus reduces the injection related local irritation. This drug complex will remain intact and is less likely to cause pain from injection if refrigerated between uses.

Other Uses

Because NK-1 is also involved in transmission of pain, there has been interest in using these antagonists for analgesia. In dogs, administration of maropitant (1 mg/kg IV, followed by continuous intravenous infusion, CRI) has decreased anesthetic requirements in dogs undergoing abdominal surgery (Boscan et al., 2011). Maropitant was shown in an uncontrolled study to decreasing coughing in dogs with chronic bronchitis. Although it decreased the severity and frequency of coughing, it had no effect on airway inflammation (Grobman and Reinero, 2016). It is presumed that the antitussive effect is through a central effect on NK-1 receptors in the cough center.

Mirtazapine (Remeron)

Mirtazapine is used more often as an appetite stimulant – especially in cats – but it also has antiemetic properties and is briefly discussed here and also included in Chapter 18, along with other appetite-stimulating drugs for cats. Mirtazapine is used as an antidepressant medication in people and is used to treat mood disorders. Its mechanism of action is unknown but it may enhance central noradrenergic and serotonergic activity. In animals it has appetite-stimulating and antinausea properties. These effects are attributed to the 5-HT_3 antagonist properties; therefore, it may share similar effects as antiserotonin medications discussed in Section Serotonin Antagonists.

Clinical Use

Mirtazapine has been studied more in cats than in dogs (Quimby and Lunn, 2013). These studies in cats demonstrated that it is an effective appetite stimulant. The dose is usually 1.88 mg per cat oral. At high doses it produces adverse effects that include vocalization and increased restlessness. In cats it has a half-life of approximately 10 hours, which allows for once-daily dosing. In cats with chronic kidney disease – for which appetite stimulation often is desired – clearance is slower and the half-life increases to 15 hours, which indicates that an every-other-day dosing schedule should be used in cats with kidney disease to avoid accumulation (Quimby et al., 2011). Mirtazapine is available as 7.5-mg tablets, but also is available in 15, 30, and 45-mg sizes. The formulation that some veterinarians prefer is a rapidly disintegrating oral tablet that dissolves easily in an animal's mouth (15, 30, and 45 mg).

Gastrointestinal Prokinetic Drugs

Prokinetic drugs increase gastrointestinal motility (Washabau and Hall, 1997). They are used in dogs, cats, horses, and occasionally ruminants to stimulate gastric emptying, rumen motility, or to increase intestinal motility (Whitehead et al., 2016). Intestinal motility is sometimes decreased after intestinal disease or surgery and can lead to ileus. Some of these drugs are intended to restore normal motility to facilitate recovery.

Metoclopramide (Reglan®, Maxeran®)

Metoclopramide has multiple actions. It is a dopamine (DA_2) antagonist, serotonin (5-HT_4) agonist and serotonin (5-HT_3) antagonist. Among the proposed mechanisms of metoclopramide is an increase in the release of acetylcholine in the GI tract, possibly via a prejunctional mechanism. It also may increase motility of gastric smooth muscle by increasing sensitivity of the cholinergic response. Since it also is a dopamine antagonist, it may antagonize dopamine's (DA_2) inhibitory action on GI motility.

Metoclopramide increases gastric emptying, increases the tone of the esophageal sphincter, and stimulates motility of the duodenum. It has less effect on distal segments of the intestine. Metoclopramide acts centrally to inhibit DA_2, which produces the antiemetic effects discussed in Section Antiemetic Drugs. In people, metoclopramide also has been used to treat hiccups and lactation deficiency.

Adverse effects from metoclopramide can include excitement (seen in horses, for example), anxiety, and involuntary muscle movements. There are also endocrine effects: There is a transient increase in prolactin and aldosterone. *Since some breast cancers are prolactin-dependent, there has been some concern about the carcinogenicity of this drug in women.*

Use in Small Animals

In dogs metoclopramide has been used as an antiemetic more commonly than other drugs. Although it has been used to promote GI motility as well, this effect is less established than previously thought (Whitehead et al., 2016). For example, it is of little benefit to increase stomach emptying in disorders of gastroparesis or chronic regurgitation. It also has been used to stimulate normal upper motility following surgery (e.g., corrective surgery for gastric dilatation), but one study showed that metoclopramide did *not* change gastric motor activity to promote gastric emptying in dogs with gastric dilatation volvulus (Hall et al., 1996). In another study, it reduced, but did not prevent gastroesophageal reflux in anesthetized dogs at a dose of 1 mg/kg (Wilson et al., 2006). Doses are in the range of 0.25 to 0.5 mg/kg, q 8–12 h, but they have been increased to 1–2 mg/kg.

Use in Horses

Some equine surgeons have used infusions of metoclopramide (0.125–0.25 mg/kg/h) added to IV fluids to reduce postoperative ileus in horses (Gerring and Hunt, 1986). It may stimulate small intestine – but not large bowel – motility, but this has little benefit for horses with intestinal ileus (Sojka et al., 1988). Undesirable side effects in horses have been common, and include behavioral changes and abdominal pain. Since this drug transiently increases prolactin secretion, there has been interest in using this drug for treating agalactia in animals, but efficacy has not been determined. Domperidone is preferred for this effect (see Section Domperidone (Motilium, Equidone)).

Use in Ruminants

The clinical use of metoclopramide in large animals has not been as common as in small animals. Metoclopramide has little usefulness in cattle, although it may increase the motility of the rumen in cattle and sheep. It has been used successfully in some cattle with functional pyloric stenosis (Braun et al., 1990), but was not effective in calves (0.1 mg/kg IM). At doses higher than 0.1 mg/kg in calves it caused severe neurological side effects (Wittek and Constable, 2005).

Domperidone (Motilium, Equidone)

Domperidone is a dopamine-2 receptor (DA_2) antagonist. It may also have α_1-receptor antagonist and serotonin ($5\text{-}HT_2$) antagonist effects. It has been available as a 10-mg tablet outside the USA as a human prokinetic drug but not allowed for human use in the USA because of cardiac toxicity. Its mechanism of action and GI prokinetic effects are similar to metoclopramide, but its efficacy has not been very impressive in animals and thus a clinical use has not been recommended (Whitehead et al., 2016). A difference between metoclopramide and domperidone is that the latter does not cross the blood–brain barrier. Therefore, adverse CNS effects are not as much of a problem compared to metoclopramide in horses. It may have antiemetic properties, but only if the stimulus for vomiting affects the CRTZ. It is capable of reaching the area postrema of the brain because this area is not protected by the blood–brain barrier. An additional effect is to stimulate lactation (see Section Use in Horses).

Use in Small Animals

The use is not reported, but it will produce a prokinetic effect in dogs at a dose of 0.05–0.1 mg/kg (2–5 mg/animal).

Use in Horses

Domperidone has been investigated for use in horses to treat fescue toxicity and agalactia. Fescue toxicosis is caused by a fungus that produces a toxin that induces reproductive toxicity in horses. The action of domperidone to increase lactation is through the stimulation of prolactin. It is approved by the FDA as an equine formulation of domperidone (Equidone oral gel, 11%). The approved dose is 1.1 mg/kg once daily starting 10–15 days prior to the anticipated foaling date. Treatment may be continued for up to 5 days after foaling if mares are not producing adequate milk. (This dose is equivalent to 5 ml per 500 kg – 5 ml per horse – daily, PO of the 11% oral gel.) Do not administer with stomach antacids such as omeprazole, cimetidine, or antacids.

The prokinetic effects in horses are not very impressive. At an IV dose of 0.2 mg/kg it was effective at restoring motility in horses with ileus, but this drug is not available in an injectable formulation. The oral absorption in horses is only 1.2–1.5%. Oral administration of 1.1 mg/kg (the approved dose) had no effect on GI function in horses but at 5 mg/kg it increased stomach emptying (Nieto et al., 2013).

Another use of domperidone is to increase digital laminar microvascular blood flow in horses. This effect is presumed to be via the action as an antagonist on vascular α_2-adrenergic receptors. It was shown to increase laminar microvascular blood flow in normal horses (1.1 and 5.5 mg/kg oral), but has not been evaluated clinically for treatment of laminitis (Castro et al., 2010).

Cisapride

In July 2000, cisapride (formerly called Propulsid®) was removed from the market because of serious cardiac adverse events, and some deaths in people, secondary to cardiac arrhythmias. The drug sponsor has no plans to market this drug to veterinarians, but there is continued interest among veterinarians and it is still available via compounding pharmacists. Until other new replacement drugs become available, such as prucalopride or mosapride, veterinarians will rely on compounded formulations or consider alternative drugs.

The reviews on cisapride by Washabau and Hall (1995) and Van Nueten and Schuurkes (1992) describe the details of its mechanism of action and clinical effects. Cisapride has greater prokinetic effects in comparison to the other drugs discussed thus far. Its mechanism is believed to be as an *agonist* for the 5-hydroxytryptamine ($5\text{-}HT_4$) receptor on myenteric neurons ($5\text{-}HT_4$ ordinarily stimulates cholinergic transmission in the myenteric neurons). (Serotonin and antagonists/agonists are covered in more detail in Chapter 19.) Cisapride may also be an *antagonist* for the $5\text{-}HT_3$ receptor. Via these mechanisms – or independently – cisapride may enhance release of acetylcholine at the myenteric plexus. There is evidence that, in cats, cisapride directly stimulates smooth muscle motility via an unknown noncholinergic mechanism (Washabau and Summarco, 1996). Cisapride increases the motility of the stomach, increases stomach emptying, and increases motility of the small intestine and colon. It accelerates the transit of contents in the bowel and intestines. Because of the $5\text{-}HT_3$ antagonist properties, it also has some antiemetic effects. Other drugs with a similar mechanism of action have been investigated, but are not in clinical use. One such drug is mosapride. Like cisapride, mosapride is also a $5\text{-}HT_4$ agonist and has been approved in some countries for treating upper gastrointestinal motility disorders in dogs (Chae et al., 2015). It has been studied in experimental horses and demonstrated to increase motility of the small intestine and cecum at a dose of 1.5–2 mg/kg PO (Sasaki et al., 2005).

Pharmacokinetics

Oral absorption is variable because of extensive metabolism. The oral absorption in dogs and cats ranges from 30 to 60%. In horses, rectal absorption has been attempted, but the amount absorbed systemically is negligible (Cook et al., 1997).

Elimination half-life is variable, but ranges from an average of approximately 5 hours in dogs and cats to a much faster rate in large animals, 2 hours or less in horses and ruminants. The volume of distribution is high in small animals (>4 l/kg) and approximately 1.5 l/kg in large animals.

Use in Dogs

In dogs at a dose of 0.1 mg/kg (range 0.08–1.25 mg/kg) orally, it stimulates smooth muscle of the stomach, small intestine, and colon, with a duration of effect of about 3 hours. Routine clinical doses have ranged from 0.1 to 0.5 mg/kg every 8–12 hours.

Although cisapride has been used by some veterinarians for treatment of megaesophagus in dogs, the response is usually poor. The canine esophagus is striated muscle, with no smooth muscle to directly respond to the medication. Clinical use in dogs has included treatment for gastroesophageal reflux, delayed gastric emptying, and small bowel motility disorders. Compared to metoclopramide, cisapride is more effective for increasing lower esophageal sphincter tone in dogs, which is helpful for preventing reflux esophagitis (Kempf et al., 2014).

Use in Cats

Experiments have demonstrated that cisapride causes stimulation of the entire GI tract in cats. Of particular interest is the effect of cisapride on colonic smooth muscle. Cisapride will stimulate this motility and has been used for treating chronic constipation. By contrast, metoclopramide has no effect on colonic smooth muscle. The dose of cisapride in cats is approximately 2.5 mg per cat, two or three times daily. Doses as high as 1 mg/kg every 8 hours, or 1.5 mg/kg every 12 hours have been recommended by some investigators (LeGrange et al., 1997).

Use in Horses

In horses cisapride increases the motility of the left dorsal colon and improves ileocecocolonic junction coordination. In contrast to metoclopramide, cisapride has fewer side effects at doses needed to affect the GI tract and greater effects on the jejunum and colon than metoclopramide. Many investigators believe that it has a place in the postoperative management of horses that have undergone abdominal surgery. One dose tested to be effective was 0.1 mg/kg, IV. At this dose, the effects appear to persist for approximately 2 hours. Oral administration is usually not possible in these horses because of gastric reflux and absorption after oral administration in a horse with gastric reflux probably is questionable.

Availability of Formulations

The previously available tablet was a 10-mg tablet from Janssen Pharmaceutica. Although cisapride is insoluble in most aqueous solutions, solubility is possible in acidic solutions. An IV form may be created by preparing a 4 mg/ml solution in tartaric acid by a reputable compounding pharmacist. The preparation of this formulation was described in the publication by Cook et al. (1997). To prepare this solution, 40 mg of cisapride is combined with 1 ml of 0.4 M tartaric acid. After the cisapride is dissolved, dilute with water to obtain a total volume of 10 ml. Oral formulations for cats have been prepared from the bulk powder administered in a capsule, via a suspension in a flavored vehicle or dissolved in cod liver oil.

Side Effects and Interactions

Adverse effects have not been reported in animals; however, abdominal discomfort has been observed when animals received high doses. In safety studies, dogs have tolerated high doses (40 mg/kg) for prolonged periods without problems.

In people, high plasma concentrations have caused cardiac arrhythmias. The arrhythmias are caused by prolonged QT intervals, presumably from blockade of potassium channels. This can lead to serious arrhythmias and has been responsible for deaths in people. These reactions have not been reported for animals. Nevertheless, one should be cautious about combining cisapride with drugs such as itraconazole and ketoconazole that may increase plasma concentrations by interfering with metabolism.

Bethanechol (Urecholine)

Many of the formulations of bethanechol have been discontinued and are no longer marketed. Some generic forms may still remain and veterinarians have also obtained it through compounding pharmacies. This drug is a cholinergic agonist that has been used to nonspecifically stimulate smooth muscle. It binds to muscarinic receptors and initiates GI smooth muscle contractions, but its actions are nonspecific. In contrast to cisapride or metoclopramide, bethanechol has a more pronounced effect on motility of the ileocecocolic region in cattle (0.7 mg/kg). In horses, bethanechol increases gastric emptying at a dose of 0.025 mg/kg IV (Ringger et al., 1996). One of its other uses has been to stimulate contraction of bladder smooth muscle in animals that have a failure to completely empty their urinary bladder when voiding. Adverse effects are common and include diarrhea and other consequences of cholinergic stimulation.

Neostigmine (Prostigmin)

Neostigmine inactivates the enzyme acetylcholinesterase, which results in inhibition of degradation of acetylcholine at the synapse. It prolongs the action of acetylcholine and may directly stimulate cholinergic receptors. It is short acting. In horses, its use is discouraged because it may actually decrease intestinal propulsive contractions, delay gastric emptying, and cause abdominal discomfort.

One of the other uses of neostigmine in animals is for the treatment of neuromuscular diseases such as myasthenia gravis. Its adverse effects are significant, and include diarrhea, salivation, respiratory difficulty, vomiting, and muscle twitching. (Usually, another anticholinesterase drug, pyridostigmine, is preferred for treating myasthenia gravis because it has fewer side effects.)

H_2-Receptor Antagonists

H_2-receptor blockers such as ranitidine and nizatidine have prokinetic effects on intestinal smooth muscle in animals. These drugs are discussed later in Section Drugs for Treatment of Gastrointestinal Ulcers in Animals.

Erythromycin

Erythromycin is a macrolide antibiotic ordinarily used to treat bacterial infections. Pharmacology of macrolides is discussed in Chapter 36. It has long been associated with vomiting and regurgitation in small animals as an adverse consequence of treatment. This effect is caused by stomach contraction and expulsion at high doses. However, at low doses it can produce a beneficial stimulation of GI motility. Not all macrolide antibiotics exhibit this property because it requires a unique chemical structure that not all drugs in this class possess. (Erythromycin has a 14 carbon structure, but other macrolides that are less effective – tylosin and tilmicosin – have a 16 carbon structure.)

Erythromycin stimulates GI motility via activation of motilin receptors, via release of endogenous motilin, or via cholinergic mechanisms in the upper GI tract (Hall and Washabau, 1997; Lester et al., 1998; Hawkyard and Koerner, 2007). Motilin is a 22 amino acid peptide released from endocrine cells of duodenal mucosa. It increases the motor contractions, the housekeeper wave, during the interdigestive period. Motility is stimulated specifically in the pyloric antrum or the smooth muscle cells of the proximal small intestine (Nouri and Constable, 2007; Nouri et al., 2008). Because most of the motilin receptors are on the stomach and proximal small intestine, there is a weak response to erythromycin in the distal GI tract. In people, erythromycin has been used to promote gastric motility and increase stomach emptying in patients with diabetic gastroparesis and used in conjunction with enteral feeding in critical care patients (Hawkyard and Koerner, 2007).

The effective dose is 1 mg/kg or less – much lower than the antibacterial dose. It was effective for stimulating motility in experimental horses (Ringger et al., 1996), but clinical responses to erythromycin in horses have been somewhat disappointing. One study showed that responses to erythromycin in horses that had undergone surgery were not as effective as the effects in healthy horses (Roussel et al., 2000). A dose of 8.8 mg/kg IM increased abomasal and rumen motility in calves (Nouri and Constable, 2007; Nouri et al., 2008; Wittek and Constable, 2005). The dose in small animals is also in the range of 0.5–1 mg/kg, but has not been tested for clinical efficacy (Whitehead et al., 2016). There is a concern that erythromycin may cause diarrhea in some horses through the effect on the normal bacterial flora of the intestine. An additional concern is that routine use may promote antibacterial resistance.

Lidocaine

Lidocaine is a well-known local anesthetic. (Local anesthetics are covered in more detail in Chapter 15, and with antiarrhythmics in Chapter 22.) It is used for local infiltration for minor surgical procedures and to treat cardiac arrhythmias. Intravenous infusions of lidocaine also improve intestinal motility in horses. Lidocaine has been used in horses postsurgically to reduce postoperative ileus. Postoperative ileus in horses is a widespread clinical problem that may be caused by (i) sympathetic stimulation, (ii) pain, or (iii) inflammation. These effects inhibit smooth muscle motility in the intestine and lidocaine may work by suppressing this transmission. Another view on the mechanism is that lidocaine does not have a direct prokinetic effect, but rather restores motility via other mechanisms (Cook and Bilkslager, 2008). These authors presented evidence that in horses lidocaine restores motility by inhibiting intestinal inflammation and reperfusion injury.

In one study (Malone et al., 2006), lidocaine administration to horses produced less reflux and shorter time of hospitalization. Infusions of lidocaine have decreased postoperative ileus either through a direct effect, or via suppression of painful stimuli. Doses in horses are 1.3 mg/kg loading dose (bolus), followed by 0.05 mg/kg/min IV infusion.

Adverse Effects

As with the other uses of lidocaine, systemic administration may produce adverse events. The most common in horses have been muscle fasciculations, ataxia, and seizures. If signs are observed, decrease rate of infusion.

Opiate Antagonists for Promoting Intestinal Motility

Opiates and their antagonists are discussed in Chapter 13. Activation of opiate μ receptors in the intestinal smooth muscle decreases propulsive motility. Expression of μ-opiate receptors have been found in the submucosal plexus, myenteric plexus, and longitudinal muscle of the ileum. Activating these receptors has been used to treat some forms of diarrhea (e.g., loperamide). Administration of opiate analgesics postoperatively (Boscan et al., 2006; Sojka et al., 1988) or increased levels of endogenous opioids (endorphins), stimulate these receptors to inhibit intestinal motility causing postoperative ileus (DeHaven-Hudkins et al., 2008). Therefore, postoperative ileus may be treated by blocking intestinal opiate receptors (μ receptors) (Hicks et al., 2004).

Selective peripheral opiate antagonists act as peripheral opioid antagonists, rather than central opioid antagonists. They do not produce a central effect because they are unable to cross the blood–brain barrier. *Naloxone should not be used for this indication because it will cross the blood–brain barrier to diminish the analgesic effect of opioids.* Such agents include alvimopan, methylnaltrexone, and naloxegol.

Alvimopan (Entereg®) has advantages over methylnaltrexone with respect to potency and duration of activity (DeHaven-Hudkins et al., 2008; Taguchi et al., 2001). It is administered orally with low bioavailability (6%) and produces a local effect on the intestine to promote motility, without diminishing analgesic effect of opioids. It is a zwitterionic molecule and the high polarity restricts its diffusion across the blood–brain barrier. A dose of 3 mg orally to people, three times daily completely reversed the GI effects of morphine, without affecting analgesia. The typical dose is 12 mg (one capsule) administered orally prior to surgery, and continuing after surgery twice daily.

Methylnaltrexone (Relistor®) is available as a SC injection (0.15 mg/kg) administered once every 48 hours for postoperative ileus. Like alvimopan, it does not have systemic effects and will not interfere with analgesia. There has been limited use of methylnaltrexone in horses. At a dose of 0.75 mg/kg IV q 12 h for four days to horses inhibited morphine-induced intestinal effects (Boscan et al., 2006).

Naloxegol (Movantik®), in 12.5 and 25-mg tablets, is a pegylated opioid antagonist. It is used for oral treatment of opioid-induced constipation. It acts peripherally because pegylation of the molecule reduces the ability of naloxegol to cross the blood–brain barrier and makes it a substrate for the efflux transporter P-glycoprotein.

Drugs for Treatment of Gastrointestinal Ulcers in Animals

Histamine H_2-receptor antagonists, sucralfate, proton pump inhibitors (omeprazole), and antacids remain the principal drugs used to manage gastrointestinal ulceration in small and large animals (Table 46.3; Figure 46.1). The medical management of ulcer diseases will not be covered in this section, but readers are referred to other references for this information (Merritt, 2003; Papich, 1993; Matz, 1995; Henderson and Webster, 2006a, 2006b; Feldman and Burton, 1990).

Table 46.3 Antiulcer drugs: clinical uses

Gastritis
Gastric ulcers
Duodenal ulcers
Gastrointestinal ulcer prevention
Esophagitis
Mast cell tumors
Hypergastrinemic syndromes
Prevention and treatment of NSAID-induced ulcers

Because many of the ulcerative diseases encountered in veterinary medicine are induced by drugs that inhibit prostaglandin synthesis (nonsteroidal antiinflammatory drugs, NSAIDs), one should be familiar with the role of prostaglandins in the GI tract, how their synthesis is inhibited, and treatments used to maintain the protective effect of prostaglandins in the GI tract. Veterinarians also should be familiar with the normal physiological role of protective mucus layer in the stomach, the cytoprotective mechanisms, role of bicarbonate secretion, and the normal mechanisms that restore epithelial cells in the stomach and intestine. These factors were reviewed by Allen et al. (1993) several years ago, but are still relevant today. When these protective factors become disrupted or compromised, ulcers can occur in animals. Gastrointestinal ulcers are a major health problem in horses, pigs, dogs, cats, and zoo animals. Conditions that increase the risk of gastrointestinal ulceration are administration of ulcerogenic drugs (NSAIDs, corticosteroids, and stomach irritants), stress, disrupted mucosal blood supply, and inflammatory diseases.

Gastrointestinal ulceration is an important medical problem in horses, in which the prevalence in animals involved in showing and racing has been listed as 81–93%, and even as high as 100% in some studies. In Thoroughbreds and Standardbreds the prevalence is was 80–95%; and in show horses it may be as high as 58%. Factors such as stall confinement, intense exercise, diet (high energy concentration in diet), and racing stress may be contributing factors. Location of ulcers in horses is primarily in the squamous epithelium (nonglandular portion). Factors that contribute to ulcers are the intermittent feeding schedule and high stomach acidity. In sick foals, ulcers also are common. Factors that contribute to ulcers in foals are NSAIDs, stress, and sepsis.

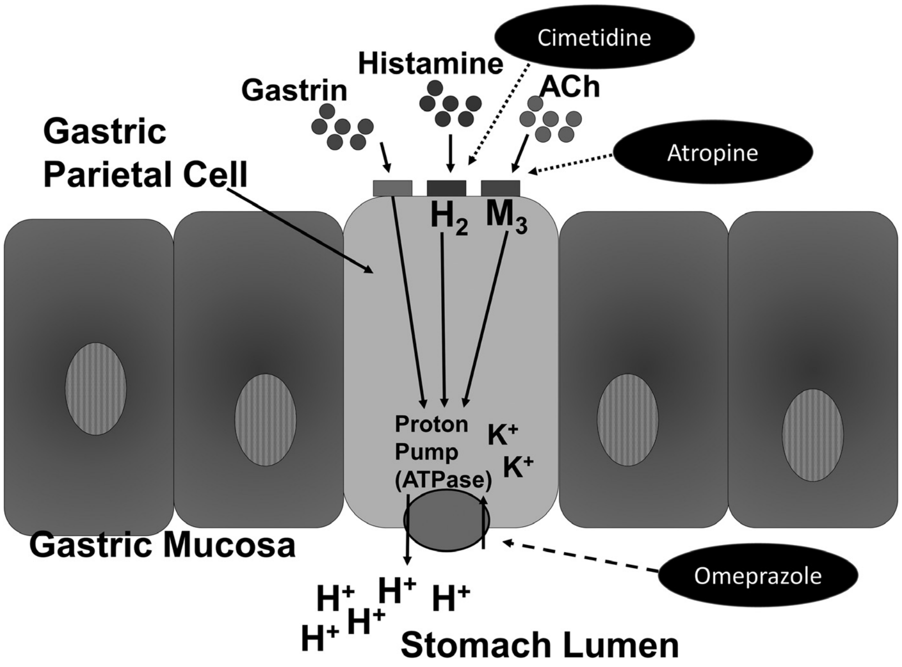

Figure 46.1 Action of stomach antisecretory drugs on various receptors that stimulate acid secretion. Action of H_2-receptor antagonists (e.g., cimetidine) and proton pump inhibitors (omeprazole) are shown. Gastrin and acetylcholine (Ach) are Ca^{++}-dependent pathways and the histamine H_2 is a cAMP-mediated pathway. See text for details.

Antacids

The common antacids are bases of either aluminum, magnesium, or calcium. Examples include aluminum hydroxide ($Al(OH)_3$), magnesium hydroxide ($Mg(OH)_2$), and calcium carbonate ($CaCO_3$, e.g., Tums®). These drugs neutralize stomach acid through a simple reaction to form water and a neutral salt. They have no systemic effects. In addition to their acid-neutralizing ability, antacids may benefit patients by decreasing pepsin activity, binding to bile acids in the stomach, and stimulating local prostaglandin (e.g., PGE_2) synthesis.

Formulations

These drugs are common and almost always available over-the-counter (OTC). Commonly available antacid preparations (e.g., Maalox®, Mylanta®, DiGel®) are combinations of magnesium hydroxide and aluminum hydroxide to optimize the buffering abilities of each compound. These combinations balance the adverse effects of constipation from aluminum hydroxide and the laxative effect from magnesium hydroxide. One of the common products is Gaviscon®, which is popular for reflux esophagitis (heartburn) in people. The regular strength tablets contain 80 mg aluminum hydroxide and 14.2 mg magnesium trisilicate. The oral liquid contains 95 mg aluminum hydroxide and 358 mg magnesium carbonate per 5 ml (mint flavor). The tablets must not be swallowed whole and should be chewed, which should be considered before using the tablets for pets.

Adverse Effects / Interactions

Adverse effects from antacids are rare because they are seldom administered long term. Additionally, antacids are not absorbed and therefore lack serious systemic effects. In animals with kidney disease, magnesium accumulation may be a problem. For example, Gaviscon® oral suspension contains 115 mg magnesium per 5 ml. Antacids will interfere with the oral absorption of other drugs (e.g., tetracyclines, fluoroquinolones, and digoxin), if administered concurrently. The magnesium component, like any divalent cation (Mg^{++}) can chelate with fluoroquinolones or tetracyclines and inhibit oral absorption. If these drugs are used together, administer the antibiotic 2 hours prior to the antacid drug. Oral antacids also reduce stomach acid, which affects oral absorption of some medications, particularly oral azole antifungal drugs. This is discussed with the drug interactions for other acid-suppressing drugs in Section Drug Interactions with Proton Pump Inhibitors.

Dosing Recommendations

Dose recommendations vary and are not precisely determined for dogs and cats. Empirically, 5–10 ml six times daily for small animals is often cited, regardless of the animal's size or product used. The frequency of administration is a significant disadvantage for administration to pets; therefore, if long-term acid suppression is needed for dogs or cats, other agents are used (see other drugs in Section Drugs for Treatment of Gastrointestinal Ulcers in Animals). Doses of 180 to 250 ml have been administered to adult horses, but only suppressed acidity for

45 min to 2 hours (Murray, 1997). A dose of 30–60 ml for calves and foals has been recommended. For the treatment of acid rumen, magnesium hydroxide has been administered orally at a dose of 225–440 grams per adult cow, or mineral oil at 4 liters per rumen. For the treatment of grain overload, antacids have been administered at a dose of 450 grams per rumen, repeated every 6–8 hours.

Histamine H_2-Receptor Antagonists

These drugs include cimetidine (the first in this class), ranitidine, famotidine, and nizatidine. These drugs became extremely popular after the initial release in the 1980s, but the use has declined because of the more potent and longer-acting proton pump inhibitors (discussed in Section Proton Pump Inhibitors). Blockade of histamine H_2 receptors inhibits gastric acidity that can be beneficial for ulcer healing in the stomach and duodenum. In animals the evidence for efficacy – based on data generated from controlled studies – is lacking. In studies that have measured stomach acidity, or compared results with proton pump inhibitors (e.g., omeprazole) the H_2-receptor antagonists were less effective. Nevertheless, many veterinarians continue to administer cimetidine, ranitidine, and famotidine with the impression that they are beneficial for the treatment of gastric and duodenal ulceration, gastric erosions, esophageal reflux disease, and gastritis in animals. There are various drugs in this group. They vary in their potency (Table 46.4) and in their pharmacokinetics, but there is no evidence for differences in efficacy among the drugs.

Mechanism of Action

Acid secretion is stimulated by three receptors (Figure 46.1): (i) gastrin receptor, (ii) histamine type-2 (H_2 receptor), and (iii) cholinergic (muscarinic) receptor (M_3). Stimulation of acid secretion occurs via stimulation of all three receptors. H_2-receptor antagonists effectively block gastric acid secretion from parietal cells by blocking the H_2 receptor.

Use in Small Animals

The use of the oldest drug in this group, cimetidine, has diminished in favor of the other drugs. Cimetidine has been administered to dogs at doses of 10 mg/kg (IV, IM, or orally), and ranitidine at doses of 2 mg/kg (IV, IM, or orally), but dosing frequency is not well-established. Famotidine (Pepcid®) has been used at 0.5 mg/kg q 12–24 h, but doses of 0.1–0.2 mg/kg q 12 h are probably equally effective. Despite the availability of pharmacokinetic information, a precise dose interval for the drugs listed has not been established because the optimum duration or extent of acid suppression for ulcer healing to occur is not known.

Table 46.4 Potency of histamine H_2 receptor blocking drugs

Drug	Relative potency
Cimetidine (Tagamet®)	1
Ranitidine (Zantac®)	4–10
Nizatidine (Axid®)	4–10
Famotidine (Pepcid®)	20–50

Use in Horses

Suppression of gastric acidity in horses was reviewed by Murray (1997). These drugs are not absorbed orally in horses as well as in dogs, with a systemic availability of only 14–30%, and the high oral doses for large animals reflect this difference (Sams et al., 1997). Cimetidine is rarely used in horses anymore because of the availability of other drugs. The dose previously used in horses was 40–60 mg/kg/day. Ranitidine has been used in horses at a dose of 2.2–6.6 mg/kg q 6–8 h (Holland et al., 1997). In clinically normal foals, ranitidine at 6.6 mg/kg, PO, or 2 mg/kg IV suppresses acid secretion for 8 hours and 4 hours, respectively (Sanchez et al., 1998).

Use in Calves

Cimetidine and ranitidine have been used in calves to maintain the pH of the abomasum >3.5 for 75% of the dosing interval (Ahmed et al., 2001). Recommended oral doses are cimetidine 100 mg/kg q 8 h, or ranitidine 50 mg/kg q 8 h.

Adverse Effects

Generally, histamine H_2-receptor antagonists have a good safety record. Adverse effects reported in people include antiandrogenic effects, decreased reproductive performance, central nervous system toxicity, and bacterial overgrowth in the stomach. These effects are rare and have not been cited as a serious problem in veterinary patients. There have been anecdotal reports of famotidine injection producing hemolytic anemia in cats. However, the conclusion from a clinical study was that when administered IV over 5 minutes, there was no risk (de Brito Galvao and Trepanier, 2008). Drug interactions are possible because cimetidine inhibits the clearance of other drugs via its action as a hepatic oxidation enzyme inhibitor (microsomal enzyme cytochrome P450 inhibitor). Ranitidine and famotidine are less likely to cause such a reaction. Drug interactions also are possible because of their effects to increase stomach pH. This can affect oral absorption of some drugs that require acidity for drug dissolution. This is discussed in more detail in Section Drug Interactions with Proton Pump Inhibitors.

Other Effects of H_2-Receptor Antagonists

Histamine H_2-blocking drugs have been used for other conditions in addition to suppressing stomach acid. Some evidence suggests that cimetidine strengthens the gastric mucosal defenses against ulceration (e.g., increases bicarbonate secretion), and enhances cytoprotection, but the relationship of these effects to ulcer healing are not well understood.

Effects on smooth muscle activity: Acid secretion inhibits stomach contractions. In experimental dogs, administration of cimetidine normalized the stomach contractions that were inhibited by stimulation of acid secretion in the stomach. Therefore, H_2-receptor antagonists may have a role in promoting an emptying of the stomach (Hayashi et al., 1990). In addition, ranitidine and nizatidine, but not cimetidine or famotidine, stimulate motility and increase gastric emptying and colonic motility via an anticholinesterase (AchE) action. Because of these motility effects, ranitidine has been used to stimulate GI motility as a prokinetic agent in animals.

Immunological effects: Cimetidine can block H_2 receptors on suppressor T lymphocytes, which increases lymphocyte responses to mitogen stimulation (Mavligit, 1987). This effect has been applied clinically in the immunological treatment of patients with tumors (Goetz et al., 1990), but the clinical benefit for most uses has been controversial. In people, the administration of cimetidine may accelerate healing of herpes lesions in immunocompromised patients.

Antiallergic effects: Histamine H_2 blockers may decrease the effects of histamine on blood vessels, and may alleviate some inflammation due to allergy. However, when used as a sole treatment, the clinical response has not been encouraging. Ordinarily when antihistamine effects are desired for treating allergic conditions, histamine type 1 (H_1) antagonists are administered.

Sucralfate (Carafate®)

Sucralfate dissociates in the acid milieu of the stomach to sucrose octasulfate and aluminum hydroxide (Figure 46.2). Sucrose octasulfate polymerizes to a viscous, sticky substance that creates a protective effect by binding to ulcerated mucosa. It protects the mucosa by preventing

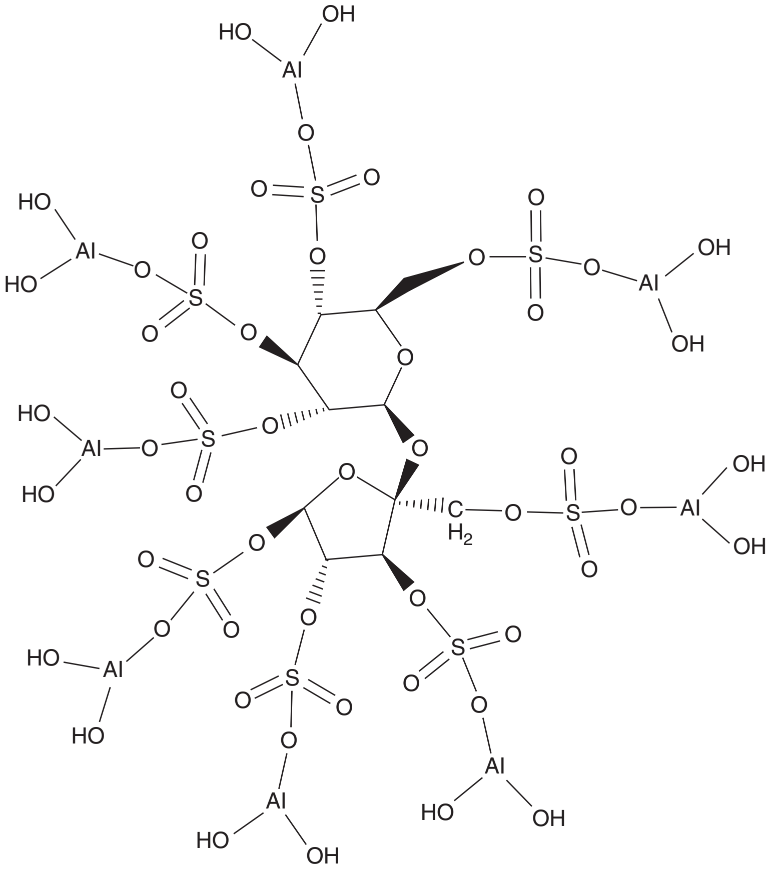

Figure 46.2 Sucralfate structure.

back diffusion of hydrogen ions and also inactivates pepsin and adsorbs bile acid. Additionally, sucralfate increases the mucosal synthesis of prostaglandins, which has a cytoprotective role and may promote healing. Increase in the epidermal growth factor may contribute to ulcer healing.

Clinical Use

Sucralfate can be beneficial for treatment of gastric ulcers in animals, but there is no evidence that it is effective for prevention of gastritis or gastric ulcers. Some veterinarians have used sucralfate to prevent ulcers caused by nonsteroidal antiinflammatory drugs (NSAIDs). Despite this common use, there is little evidence of its efficacy for preventing NSAID-induced ulcers. Because sucralfate is not absorbed, it is virtually free of adverse effects. The most common side effect associated with its use in people has been constipation. Dosage regimens for sucralfate have been extrapolated from human dosages. Doses administered are as follows: 250 mg (one-quarter tablet) to cats two to three times daily, 500 mg to 1 gram to dogs, and 4 grams orally to foals. The tablets must disintegrate to produce a therapeutic effect. In studies performed in dogs, there is evidence that the intact tablets do not disintegrate and crushing the tablets and administering as a liquid suspension may be more effective (KuKanich and KuKanich, 2015).

Drug Interactions

Even though sucralfate has some "coating action," this does not prevent oral absorption of drugs from the intestine. However, sucralfate contains aluminum and may interfere with absorption of some drugs. For example, aluminum will chelate fluoroquinolone and tetracycline drugs and inhibit oral absorption (KuKanich and KuKanich, 2015; KuKanich et al., 2016). However, the effects on fluoroquinolone absorption in dogs are inconsistent (KuKanich et al., 2016). If these antibiotics are needed, administer them first, 30 minutes to 2 hours prior to sucralfate administration.

Table 46.5 Proton pump inhibitors (PPI)

Omeprazole (Prilosec®) 20 mg capsule and other forms
Lansoprazole (Prevacid®) 20 mg tablet
Rabeprazole (Aciphex®) 20 mg tablet and IV
Pantoprazole (Protonix®) 40 mg tablet, and IV
Esomeprazole (Nexium®) 20, 40 mg capsules
Dexlansoprazole (Kapidex®) 30, 60 mg capsules

Proton Pump Inhibitors

Gastric acid secretion is stimulated by hormonal and neural stimulation of parietal cells, which are located in the proximal stomach (Figure 46.1). The proton pump inhibitors (PPI; omeprazole, pantoprazole, esomeprazole) (Table 46.5) are substituted benzimidazole drugs that target the final common pathway of acid production. These drugs have emerged as more effective and widely used than the previously discussed histamine type-2 antagonists (Barkun et al., 2012). The PPIs are weak bases that are unprotonated and act as prodrugs in the physiological pH of the blood. After systemic absorption, they become trapped in the acidic parietal cells of the stomach (Figure 46.3). Once the PPI diffuses and accumulates in the acidic environment of the secretory canaliculi of the active parietal cell (up to 1000 times the plasma concentration), it becomes protonated and trapped, where it forms disulfide bonds with cysteine residues on the alpha subunit of the H^+-K^+-ATPase enzymes, resulting in prolonged to irreversible enzyme inactivation (Kromer et al., 2000). The effect is prolonged because acid secretion resumes only after new proton pumps are synthesized in the parietal cells. There may be an initial delay before achieving maximal efficacy. Previously dormant parietal cells are activated following initial PPI administration. Thus, inhibition of acid secretion is approximately 30% of maximal on day 1 of PPI administration since not all H^+-K^+-ATPase enzymes are bound by drug. Maximal inhibitory effect is thought to be achieved within

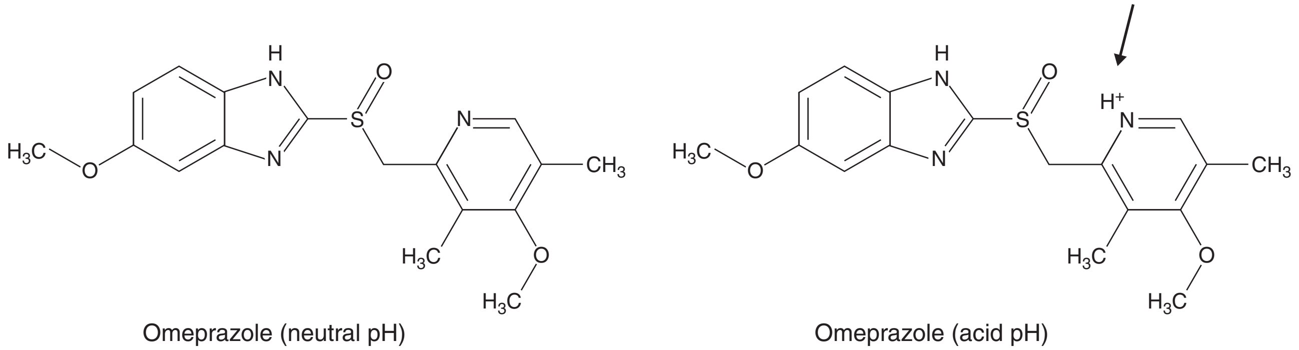

Figure 46.3 Omeprazole structure. At neutral pH the structure has no charge, but after entering the acidic parietal cell, it picks up a positive charge (shown by arrow), to "trap" the drug in the cell.

approximately 2–4 days of PPI administration (Kromer et al., 2000; Larsson et al., 1983; Abelo et al., 2002; Wallmark et al., 1985). Because the inhibitory activity of a PPI is dependent on its ability to accumulate in the parietal cell and bind to active H^+-K^+-ATPase enzymes, the plasma concentrations of PPIs do not necessarily predict their efficacy. The best surrogate predictors of the inhibitory effect of PPIs on gastric acid secretion are the area under the concentration–time curve and gastric pH profile (Andersson et al., 2001).

The delay in the maximum activity of PPI is also related to the stability of these drugs in the stomach. They are acid-labile drugs and when administered in an acid environment, the drug may be degraded before it reaches the intestine where it can be absorbed systemically. After repeated administration, acid secretion gradually diminishes, thus maximizing the amount of active drug absorbed from the intestine (Prichard et al., 1985). Initial treatment with intravenous formulations (e.g., pantoprazole), administering an enteric-coated delayed-release formulation, or combining oral formulations with bicarbonate (Zergid® is an example of a combination of omeprazole and sodium bicarbonate) will decrease the lag-time for achieving maximum effect. Another theory to explain the increased concentrations with repeated doses is an inhibition of the cytochrome P450 enzymes by omeprazole, thus inhibiting its own metabolism (Andersson et al., 1990). Omeprazole is a well-known inhibitor of the enzyme CYP 2C19 in people, but it is not known which enzymes (if any) are inhibited in dogs or cats.

Pharmacokinetics and Metabolism

Cytochrome P450 (CYP) enzymes are responsible for the metabolism of the majority of the PPIs. The exception being rabeprazole, which is unique among the PPIs in that the CYP system is only responsible for 15–20% of its metabolism (Yasuda et al., 1995). Genetic polymorphism in CYP genotypes is a well-established cause for the large interindividual and interethnic variation in the pharmacokinetics and pharmacodynamics of PPIs in humans (Egan and Murray, 2000). There are differences in response among animals, but not enough work has been done to determine if this is caused by differences in CYP expression among animals. The pharmacokinetics have been extensively studied in horses (Sykes et al., 2014, 2015a, 2015b, 2016). Differences in pharmacokinetics exist for various formulations, but these differences may not account for clinical efficacy differences. More discussion of the formulations is provide in the Section Clinical use and efficacy in horses.

Clinical Use

Acid secretion is activated by ingestion of a meal. Therefore, it is recommended to administer most PPIs 30 minutes to 1 hour prior to a meal to minimize the effects of stomach acidity on oral absorption, and to ensure that maxim absorption occurs so that the drug is in circulation and available to accumulate in the parietal cell prior to meal-stimulated acid secretion. If administered once daily, morning administration is recommended because the prolonged fast results in higher number of H^+-K^+-ATPase enzymes available to mobilize to the secretory canaliculi (Chiverton et al., 1992). Newer PPIs such as the R enantiomer of lansoprazole, dexlansoprazole, are designed to be slow release and effective against both basal and meal-stimulated acid secretion, thus gastric pH may not be impacted by the timing of meal intake and administration of newer PPIs (Lee et al., 2009). There is evidence in humans that these drugs are superior to other antisecretory drugs such as histamine H_2-antagonists for treatment and prevention of ulcers caused by NSAIDs. In human studies, omeprazole has had better healing rates in comparison to other antiulcer drugs – such as H_2-receptor blockers – for some types of ulcers and esophagitis. In addition, these drugs have an inhibitory effect on the bacteria *Helicobacter pylori*, which is a cause of gastritis and ulcers in people.

Clinical use and efficacy in dogs and cats: Despite their widespread use in companion animals, there have been limited clinical studies investigating the efficacy of PPIs in companion animals. Far more clinical efficacy studies exist for horses, where they have FDA-approved products in the USA and other countries (Sykes et al., 2015a, 2015b). The majority of canine and feline PPI studies were designed as preclinical development trials prior to study of these drugs in humans. Many of these studies were performed following pharmacological stimulation of gastric acid secretion using healthy animals with surgically placed gastric fistulas. It is unknown if results from these studies accurately reflect the response of intragastric pH to PPIs in clinical canine and feline patients. Comparison studies performed in healthy dogs and cats with neither of these interventions demonstrated that PPIs are superior to histamine H_2 antagonists for raising and maintaining high intragastric pH and for prevention of exercise-induced gastritis in dogs (Bersenas et al., 2005; Tolbert et al., 2011; Parkinson et al., 2015; Sutalo et al., 2015). A typical human dose of omeprazole is 20 mg. A full 20-mg capsule has been administered to dogs, regardless of weight. Alternatively, doses of 0.7–1 mg/kg administered once or twice daily has been used most often. Early studies in dogs and cats used once-daily administration, but results from other studies in healthy dogs and cats suggest that omeprazole should be administered twice daily to achieve pH goals established for the treatment of acid-related disorders in people (Bersenas et al., 2005; Tolbert et al., 2011; Sutalo et al., 2015; Burget et al., 1990; Bell et al., 1992). There have been no benefits demonstrated for the coadministration of histamine H_2 antagonists and PPI in animals (Tolbert et al., 2015).

Clinical use and efficacy in horses: The use in horses is widespread because as discussed in Section Drugs for Treatment of Gastrointestinal Ulcers in Animals, performance horses have a high incidence of gastric ulcers. Omeprazole is most often used because of approved formulations developed for horses (Sykes et al., 2015a, 2015b, 2016). A dose of 1 mg/kg/day is needed to inhibit acid secretion (Andrews et al., 1999) and 1.4–2 mg/kg is needed for a more consistent effect (Baker and Gerring, 1993; Murray, 1997). A dose of 4 mg/kg PO suppressed acid secretion in foals for 22 hours. Omeprazole (4 mg/kg PO, q 24 h) was significantly better than ranitidine (6.6 mg/kg PO, q 8 h) for healing ulcers (Lester et al., 2005). Omeprazole is approved for this use as GastroGard®, an oral paste for horses at a dose of 4 mg/kg once daily for 4 weeks, and for prevention of ulcers the dose is 2 mg/kg once daily. Three to five days of administration are required for maximal suppression of acid secretion. To promote ulcer healing in horses, the pH of the stomach should be maintained above 3.5 (Andrews et al., 1999). An over-the-counter (OTC) form of this drug for horses is also available (UlcerGuard®). The dose and concentration is the same as the prescription drug (GastroGard®). If compounded formulations are prepared – especially if prepared from a bulk chemical substance – degradation may occur in the stomach, which renders the drug ineffective (Nieto et al., 2002). Plain, uncoated omeprazole made from powder had half the bioavailability compared to the commercial formulation (Sykes et al., 2015b).

Although GastroGard®is the most common formulation, other formulations have become available for horses in some countries. Two buffered formulations are available in other countries, Omoguard® and Abgard,® and two enteric-coated granule formulations, Gastrozol® and BOVA Omeprazole Granules.® These other formulations were described, and evaluated by Sykes et al. (2015a, 2015b, 2016) (see Table 1 of Sykes et al., 2016 for descriptions). When four formulations were compared to the reference GastroGard®, there were no statistical differences in pharmacokinetics for three formulations but one (Abgard®*)* produced a lower peak concentration.

The same research group also evaluated omeprazole doses used in horses (Sykes et al., 2014, 2015a, 2015b). They found that 1 mg/kg oral per day was therapeutically equivalent to 4 mg/kg once daily for treatment of naturally occurring gastric ulcers in Thoroughbred race horses. They also found that gastric ulcers that occur in horses have lower healing rates from omeprazole if they occur in the glandular portion of the stomach compared to the squamous (nonglandular) portion of the stomach.

Adverse Effects

Despite easy access without prescription (OTC) of these products for humans and widespread use, adverse reactions are rare. Few adverse effects have also been reported with PPI use in dogs, cats, and horses. One explanation is that the effects on the gastric H^+, K^+-ATPase are not shared by other ATPases. Nongastric ATPases lack the ability to convert the prodrug to the active form, which requires an acid environment. Thus, the PPI accumulate in the acidic parietal cells of the stomach, but much less so in other cells of the body.

Two adverse consequences as a result of chronic administration of PPI have received attention. Because of loss of negative feedback mechanisms, gastrin levels are elevated as a result of PPI administration (Solcia, 1993). Consequently, persistently elevated gastrin levels exert a trophic effect on the gastric mucosa. This property has been linked to development of gastrin neoplasia in laboratory animals, specifically tumors arising from enterochromaffin-like cells in rats. This was a significant concern when these agents first became popular in the 1980s. However, after years of experience with PPI in people, this has not been demonstrated to be a realistic concern. One study has demonstrated elevated gastrin levels in cats after chronic administration (Gould et al., 2016). However, other than a transient rebound hypersecretion of acid in some of the research cats, no other adverse effects were identified. No similar studies have been done in dogs.

The second effect often discussed as a result of chronic administration of PPI is small intestinal bacterial overgrowth (SIBO) (Lo and Chan, 2013). Proton pump inhibitors increase the population of bacteria in the small intestine (Wallace et al., 2011). By increasing the stomach pH, this can potentially increase colonization of bacteria that would otherwise have been inhibited in the acid milieu of the stomach and upper small intestine. Increased survival of swallowed bacteria in the upper GI tract is influenced by other effects of PPI such as decreased intestinal peristalsis, decreased stomach emptying, changes in epithelial mucus composition, and increased bacterial translocation. Bacteria overgrowth in the small intestine can lead to some malabsorption syndromes and impaired vitamin B_{12} absorption. Increased growth of bacteria in the upper GI tract could also increase risk of more severe consequence in small animal patients with aspiration pneumonia. Studies in dogs have shown that omeprazole at a dose of 1.1 mg/kg twice daily for 15 days increased total bacteria in the duodenum (*Lactobacillus*) and a decrease in stomach *Helicobacter* spp. during omeprazole treatment (Garcia-Mazcorro et al., 2012). However the overall phylogenic composition of the gastric and duodenal microbiota did not change.

Bacterial overgrowth also can have consequences when the PPI are administered with NSAIDs. This has been a concern in people because these drugs are often prescribed together in patients at risk for upper GI injury from nonsteroidal antiinflammatory drugs (NSAIDs) (Marlicz et al., 2014). Whether or not this drug combination produces a risk in dogs, cats, or horses has not been determined.

Diarrhea is the most common adverse effect reported in association with PPI administration in dogs (Bersenas et al., 2005; Davis et al., 2003). Adverse effects have not been observed in cats. After prolonged twice-daily administration for 60 days of omeprazole to experimental cats, the study did not identify significant problems (Gould et al., 2016). There were no significant changes in magnesium, cobalamin, calcium, bone mineral density, or bone mineral content.

Drug Interactions with Proton Pump Inhibitors

Several documented, and potential, drug–drug interactions have been associated with PPI administration. The administration of PPI raises the gastric lumen pH from a normal of 1–2 units, to above 4 and sometimes higher. Some drugs and nutrients require acid for oral absorption because they exhibit pH-dependent drug solubility or dissolution. Some drugs require acid pH to release the drug from a protective coating. Therefore, administration of PPI simultaneously with some drugs may affect oral absorption, reducing the systemic exposure and clinical effect. The drugs affected by dissolution are primarily the weak bases. These drugs require acid pH to increase solubility, which is the first step in GI drug absorption (after product disintegration).

Antifungal drugs of the azole class include ketoconazole, itraconazole, and new agents voriconazole and posaconazole (discussed in Chapter 38). These drugs are inherently poorly soluble. For oral absorption to occur these drugs must undergo dissolution, which only occurs at a low pH. Subsequently, they must be administered orally with food to stimulate acid secretion. This effect was best demonstrated by the significant impairment of dissolution of ketoconazole at a high pH, and the decreased oral absorption of ketoconazole in experimental dogs caused by raising the stomach pH profile (Zhou et al., 2005).

Other drugs that may have impaired oral absorption from an increase in stomach pH include iron and mycophenolate. Hydrochloric acid in the stomach promotes iron absorption because it reduces the ferric acid form to the more soluble ferrous form. Human patients on chronic PPI therapy may have reduced oral absorption of iron. Treatment with PPI is considered in human patients with hereditary hemochromatosis in order to reduce the need for frequent phlebotomy (Hutchinson et al., 2007). The effect of chronic administration of PPI on oral iron absorption has not been explored in dogs, cats, or horses. Mycophenolate mofetil (discussed in Chapter 45) requires acid pH for dissolution of the medication and for hydrolysis to mycophenolic acid. If PPI are administered concurrently, the increased pH reduces the absorption of the active drug (Gabardi and Olyaei, 2012). This interaction has been demonstrated in people, but has not been explored in dogs or cats.

Drug interactions that affect drug metabolizing enzymes: PPI are metabolized by hepatic cytochrome P450 enzymes, and drug interactions with CYP-dependent drugs are possible because of the inhibitory effect of PPIs on the CYP system. The predominant enzyme identified in people is CYP2C19. Administration of omeprazole can inhibit its own metabolism (discussed in Section Proton Pump Inhibitors) or inhibit metabolism of other drugs metabolized by the same enzyme (Furuta et al., 2001; Mullin et al., 2009; Gerson and Triadafilopoulos, 2001). Drugs affected in people include warfarin, clopidogrel, and diazepam. The population of CYP 450 enzymes are not the same in people and dogs (Court, 2013); therefore, it is undetermined if omeprazole inhibits metabolism of these, or other drugs, in dogs.

Of interest is the effect of omeprazole on the conversion of clopidogrel (Plavix®) to the active form. Clopidogrel is a prodrug and, after administration, must be converted to the active form prior to inhibiting platelet function (discussed in Chapter 25). In people, omeprazole may inhibit one of the enzymes responsible for conversion. Concurrent administration of omeprazole and clopidogrel may interfere with the biotransformation that is responsible for the formation of the active metabolite of clopidogrel and compromise antiplatelet therapy (Laine and Hennekens, 2010). The US Food and Drug Administration (FDA) recommends that "concomitant use of drugs that inhibit CYP2C19 (e.g., omeprazole) should be discouraged." However, a study in dogs showed that, coadministration of omeprazole with clopidogrel to experimental dogs did not diminish the antiplatelet effects of clopidogrel (Thames et al., 2017). These effects have not been studied in cats or horses.

Synthetic Prostaglandins

Synthetic prostaglandins – administered orally – produce many of the protective effects on the GI mucosa of natural prostaglandins (Allen et al., 1993; Henderson and Webster, 2006a, 2006b). Misoprostol (Cytotec®) is a synthetic PGE_1 analogue that has been used in people to diminish the risk of ulcers associated with NSAID therapy. In dogs, it prevents duodenal hemorrhage and ulceration associated with aspirin therapy, but has not been tested with other NSAIDs in small animals. Misoprostol is helpful for preventing GI ulcers, but is less beneficial for treating ulcers once they occur. Its use appears to be limited to NSAID therapy. Misoprostol was *not* effective for preventing gastritis, ulcers, and gastrointestinal hemorrhage associated with corticosteroid administration (Hanson et al., 1997).

Use in Small Animals

Misoprostol is available as 0.1 and 0.2-mg tablets. Dosages for dogs range from 2 to 5 μg/kg. It is absorbed and rapidly metabolized to an active metabolite. At high doses (>5 μg/kg) adverse effects of diarrhea have been reported.

Use in Horses

Misoprostol has been shown to decrease stomach acidity in horses. However, misoprostol may cause abdominal discomfort, cramping, and diarrhea. There have been anecdotal benefits for treating or preventing right dorsal colitis in horses associated with phenylbutazone treatment. However, more safety and efficacy data are needed before the clinical use is established.

Nongastrointestinal Uses for Misoprostol

Misoprostol was shown to have modest antiinflammatory effects and has been effective for some dermatoses in dogs. In these studies, 3–6 μg/kg was administered to dogs with atopic dermatitis three times daily orally. Misoprostol produced a decrease in pruritus and clinical score of 30%. Side effects may prevent more widespread use and higher doses.

Misoprostol is an effective abortifacient. It has been used as a component of medical treatment of abortion in humans, in combination with other drugs. The protocol in people is 200 mg mifepristone (RU-486) oral, followed by 800 mg misoprostol 24–48 hours later. This protocol has not been used in animals, but if it is administered inadvertently to a pregnant animal, abortion could occur.

Drugs Used to Treat *Helicobacter* Gastritis

Treatment of gastritis and ulcers caused by the bacteria *Helicobacter pylori* and *Helicobacter*-like organisms has been used in animals that have not responded to other treatments. These organisms have been identified in biopsy specimens from dogs and cats, but its role in gastritis and ulcers has yet to be established (Yamasaki et al., 1998). Some studies have found no association between *Helicobacter* infection and gastritis (Winberg et al., 2005; Haponen et al., 1998). However, in some animals with gastritis, treatment has helped decrease signs when treatment was initiated with ampicillin, metronidazole, and famotidine. A discussion on the role of *Helicobacter* as a cause of gastritis in animals can be found in the articles by Simpson et al. (2000) and Neiger and Simpson (2000). One of the authors recommends that animals with clinical signs of gastritis and positive for *Helicobacter* or *Helicobacter*-like organisms should be treated. The oral treatment for animals that has been the most widely used is a 2-week course of the combination of metronidazole and/or clarithromycin, plus amoxicillin, and a proton-pump inhibitor, or H_2-receptor antagonist.

In people, *Helicobacter pylori* gastritis has been treated with the simultaneous use of metronidazole, omeprazole, and clarithromycin (Suerbaum and Michetti, 2002). One regimen uses clarithromycin (Biaxin®) and omeprazole (Prilosec®). A product called Tritec® combines ranitidine with bismuth citrate and is designed to be administered concurrently with clarithromycin (Biaxin®). Some regimens also use amoxicillin or metronidazole. Some synergism is achieved with these combinations. For example, H_2 antagonists or omeprazole enhance the antibacterial activity of metronidazole and perhaps other antibiotics.

Drugs for Treatment of Diarrhea

The most important treatment for diarrhea should include fluid therapy, electrolyte replenishment, maintaining acid/base balance, and control of discomfort. But drugs such as antimicrobials, motility modifiers, and intestinal protectants are sometimes used. Many of the causes of diarrhea do not require drug therapy and the disease is self-limiting. In some instances, treatment may be needed to temporarily relieve clinical signs. Some of these treatments are discussed here. Diarrhea caused by intestinal parasites is covered in Chapters 39–42 of this book.

Mucosal Protectants and Adsorbents

Kaolin–Pectin Formulations

Products that are promoted as mucosal protectants such as mixtures of kaolin and pectin (Kao-Pectate®) have been popular. Kaolin is a form of aluminum silicate and pectin is a carbohydrate that is extracted from the rinds of citrus fruits. The manufacturers claim that kaolin–pectin acts as a demulcent and adsorbent in the treatment of diarrhea. The action of kaolin–pectin is believed to be related to binding of bacterial toxins (endotoxins and enterotoxins) in the GI tract. However, experimental studies have shown that kaolin–pectin is an ineffective binder of *E. coli* enterotoxin and clinical studies have failed to show a benefit from the administration of kaolin–pectin. This product may change the consistency of stools, but it will not decrease fluid or electrolyte loss, nor will it shorten the duration of illness.

Kao-Pectate formulations contain salicylate as one of the active ingredients. There is 8.7 mg/ml in the *Regular*

Strength, and 16 mg/ml in the *Extra Strength* formulation. Since some animals may be sensitive to salicylates, the salicylate content of the formulation should be considered before administering the product to animals.

Clinical use of kaolin–pectin: Despite the lack of clinical evidence of efficacy, some veterinarians administer this drug at a dose of 1 to 2 ml/kg, q 6 h. Kaolin–pectin is not absorbed, but salicylate may be absorbed in most animals (see Section Bismuth Subsalicylate, and Papich et al., 1987). Drug interactions are also possible. Kaolin–pectin may adsorb or bind other drugs that are administered orally and decrease their effectiveness.

Bismuth Subsalicylate

The most common form of bismuth subsalicylate is Pepto Bismol®. Although other "mucosal protectants" may have questionable efficacy, this product is considered by many gastroenterologists to be a useful symptomatic treatment for acute diarrhea. Its efficacy has been proven in controlled clinical trials in humans with acute diarrhea, particularly the form caused by enterotoxigenic *E. coli* (also known as traveler's diarrhea) (Bierer, 1990). Bismuth may have some ability to adsorb bacterial enterotoxins, and it may produce some gastric or intestinal protective effect. The salicylate component may be responsible for efficacy, because it produces an anti-inflammatory action. There are five sources of salicylate in this formulation accounting for approximately 9 mg of salicylate per ml. Two tablespoons (the typical human dose) contains almost 300 mg of salicylate. An "extrastrength" formulation contains twice this amount. Practically all of the salicylate from PeptoBismol® is absorbed systemically when administered to dogs and cats (Papich et al., 1987). Despite the systemic absorption, the amount is unlikely to produce salicylate toxicity. Even in cats – a species sensitive to salicylate toxicity – it is unlikely that enough would be administered from standard dosages to produce a toxic reaction.

Clinical use of bismuth subsalicylate: The dose administered to animals was not derived from clinical studies, but extrapolated from human dosages. One to three ml/kg/day in divided doses have been administered safely (even to cats). Some animals may not like the taste, but otherwise there have not been serious adverse effects reported from administration of PeptoBismol®. Salicylate toxicosis is possible from overdoses, and pet owners should be advised to limit the amount administered, especially in animals with other primary diseases that may have initiated the diarrhea. Pet owners should be warned that PeptoBismol® may turn the stools black. Bismuth also has an anti-*Helicobacter* effect in the stomach, and has been included in many of the regimens for treating *Helicobacter* gastritis. Therefore some benefits for treating gastritis may have been caused from the action on *Helicobacter* or *Helicobacter*-like organisms.

PeptoBismol® also has been administered for the treatment of acute diarrhea in large animals, especially foals and calves. The presumed benefit is from binding to enterotoxins or from the antiprostaglandin effect.

Motility Modifiers for Treatment of Diarrhea

Anticholinergic Drugs (Antimuscarinic Drugs)

Anticholinergic drugs significantly decrease intestinal motility and secretions and have been included as ingredients in antidiarrheal preparations. These drugs inhibit muscarinic action via their antagonism of either the muscarinic M_1 or M_3 receptor. (M_1 is a ganglionic receptor, and M_3 acts at peripheral sites.) Their parasympatholytic effects decrease segmental and propulsive intestinal smooth muscle contractions. Although they do not alter the course of the disease, anticholinergic drugs may decrease the urgency associated with some forms of diarrhea, decrease the fluid secreted into the bowel, and decrease abdominal discomfort associated with hypermotility. The limitation for the use of anticholinergic drugs lies in the questionable efficacy for most diarrhea seen in veterinary medicine (few cases of diarrhea actually can be classified as "hypermotile"). These drugs are also associated with important side effects. Anticholinergic drugs are discussed in more detail in Chapter 8.

Efficacy: Although the anticholinergic agents clearly decrease intestinal motility, there is no clear evidence that a decrease in motility is always beneficial. Intestinal motility is already impaired in many patients with some forms of diarrhea and these drugs may actually worsen the diarrhea by creating a "stovepipe" effect. The use of these drugs should be avoided if the diarrhea is infectious (e.g., caused by *Salmonella*).

Adverse effects: Anticholinergic drugs have profound systemic pharmacological effects. If they are administered in sufficient doses to affect the bowel, adverse effects are possible, which include ileus, xerostomia, urine retention, cycloplegia, tachycardia, and central nervous system (CNS) excitement. Chronic administration can lead to serious bowel atony. In addition to decreasing bowel motility, anticholinergic drugs decrease stomach emptying, which can lead to stomach distention and discomfort. This is certainly contraindicated in a patient that has gastritis and is vomiting. Rumen atony is possible in cattle.

Examples of drugs: Atropine is the best known anticholinergic drug, but because it has many other systemic

Table 46.6 Anticholinergic drugs used for treatment of diarrhea

Tertiary amines
atropine
scopolamine
Quaternary amines
methscopolamine
isopropamide
propantheline
glycopyrrolate

effects, it is not ordinarily used for its antidiarrheal properties. To avoid CNS effects, it is desirable to administer drugs that are quaternary amines. Because these drugs are charged, their lipophilicity is reduced and they do not cross the blood–brain barrier as readily as tertiary amines (Table 46.6).

Quaternary amines used in veterinary medicine (Table 46.7) include methscopolamine, propantheline (Pro-Banthine®), and isopropamide (Darbid®). Darbazine®, an old combination rarely available today, contains both isopropamide and prochlorperazine. Anticholinergic drugs have been included in formulations such as aminopentamide in Centrine® and diphemanil in Diathal®. Combination products such as Donnatal® contain scopolamine, atropine, and hyoscyamine (in addition to phenobarbital). Some of these formulations are outdated and unavailable today.

N-Butylscopolammonium bromide (Buscopan): N-butylscopolammonium bromide is an anticholinergic drug used to treat colic in horses (0.3 mg/kg IV). N-butylscopolammonium bromide is an antispasmodic drug approved for treatment of colic associated with spasm of the intestine in horses. For other forms of colic, inhibition of intestinal motility may be contraindicated. Another use of N-butylscopolammonium bromide in horses is to relax the large bowel to facilitate rectal examination and colonoscopy.

When used to treat spasmodic colic, within 5–10 minutes of IV injection, spasm of intestinal smooth muscle is relieved and decreased intestinal smooth muscle contraction occurs. The duration is short (20 minutes). Adverse effects from N-butylscopolammonium include other anticholinergic effects such as transient increase in heart rate lasting for approximately 30 minutes. It should not be used in horses with an impaction or in horses with ileus.

Table 46.7 Antidiarrhea drugs and doses

Drug	Dose
Opiates	
Codeine	0.5–1.0 mg/kg, PO, q8–12h
Morphine	0.05–0.1 mg/kg, IM, SC, q8–12h
Diphenoxylate (Lomotil®)	0.1–0.2 mg/kg, PO (dog), q8–12h or 0.05–0.1 mg/kg, PO (cat), q12h
Loperamide (Imodium®)	0.1–0.2 mg/kg, PO, q8–12h
Paregoric	0.05–0.06 mg/kg, PO, q12h
Anticholinergic drugs	
Propantheline (Pro-Banthine®)	0.25–0.5 mg/kg, PO, q8–12h
Aminopentamide (Centrine®)	0.01–0.03 mg/kg, q8–12h or 0.1 mg/cat PO, IM, SC, q8–12h
Diphemanil (Diatha®l)	1.8 mg/kg, IM, q12h

Opioids

Opioids can have both antisecretory and antimotility actions as they act on the μ (mu)-opiate receptors of the GI tract. They decrease propulsive intestinal contractions and increase segmentation (an overall constipating effect) (DeHaven-Hudkins et al., 2008). They also increase the tone of GI sphincters. In addition to affecting motility, opiates have an antisecretory effect and stimulate absorption of fluid, electrolytes, and glucose. Their effects on secretory diarrhea are probably caused by inhibition of calcium influx and decreased calmodulin activity (Hedner and Cassuto, 1987). These drugs can affect small animals and have been documented to inhibit colonic motor activity in horses (Roger et al., 1985; Boscan et al., 2006).

Examples of Drugs

The full range of opioid and opiate drugs are discussed in Chapter 13. The constipating effects of morphine and other opioids are well known. However, it would be irrational to administer morphine repeatedly to animals simply for the antidiarrhea effect. Instead, opioids are used that produce little systemic effect. Two synthetic opioids are known for their specificity of action on the GI tract and their effect on secretory diarrhea. They are primarily active locally on the opiate μ-receptors and they produce GI effects without other systemic side effects. Two such compounds are *diphenoxylate* (Lomotil®) and *loperamide* (Imodium®) (Johnson, 1989). Diphenoxylate is a derivative of meperidine and not commonly used today. Diphenoxylate (Lomotil®) contains 25 μg of atropine per 2.5 mg tablet. The amount of atropine contained in this mixture may contribute to some inhibition of intestinal smooth muscle, but is present in the formulation primarily to discourage abuse by people.

Loperamide is the most often used and is available OTC in capsules (Imodium®) of 2 mg and an oral solution of 0.2 mg/ml (for dosages, see Table 46.7). The typical dose for small animals is 0.1 mg/kg, q 12 h, PO. Paregoric is an older compound (tincture of opium), found in many antidiarrheal products; 5 ml of paregoric corresponds to approximately 2 mg of morphine.

Adverse Effects

Opiates can have potent effects on the GI tract and should be used cautiously. Loperamide is an over-the-counter drug available for people, often administered to animals. These drugs are generally contraindicated in infectious diarrhea because opiates may significantly slow GI transit and increase the absorption of bacterial toxins.

Loperamide ordinarily does not cause central nervous system opiate-related effects because the membrane transporter p-glycoprotein (p-gp) helps to remove this drug from the central nervous system. However, dogs deficient in p-gp (e.g., Collie dogs and other herding breeds) may be prone to toxicity (Mealey, 2004). Consult Chapter 50 for further discussion.

Antimicrobial Therapy for Treatment of Diarrhea

The routine use of antimicrobials to treat diarrhea has been questioned by most gastroenterologists. In most cases of diarrhea in small animals, a bacterial etiology cannot be identified. In large animals diarrhea is often caused by bacteria (enterotoxigenic *E. coli*, for example), but antibiotic therapy may not alter the course of the disease and there is a lack of well-controlled studies to demonstrate efficacy. Nevertheless, oral nonabsorbed antibiotics are sometimes combined with motility modifiers, adsorbents, and intestinal protectants in commercial preparations. Some of these are irrational combinations. Administration of oral antibiotics can actually be a *cause* of diarrhea (Bartlett et al., 2002). More detailed descriptions of the antimicrobials are provided in Chapters 32–37 and 42 of this book. Some examples where antibiotics may be indicated in animals are listed here.

Antibiotic-Responsive Enteropathy in Small Animals

This syndrome has been previously known by different names as *small intestinal bacterial overgrowth (SIBO),* or different forms of antibiotic-responsive intestinal diseases. Now they tend to be grouped together in a larger category of antibiotic-responsive enteropathies.

Escherichia coli *and* Clostridium*:* *Clostridium* spp. is a fastidious gram-positive anaerobic, spore-forming bacillus. Treatments that have been used include antimicrobial treatment and administration of probiotics. When antibiotics are considered, one should administer an oral drug that is active in the GI lumen. The antibiotic administered should have activity against anaerobic bacteria and may include metronidazole, ampicillin, a macrolide, or clindamycin. Vancomycin is used orally in people when resistance to other drugs is suspected.

For intestinal infections caused by *E. coli*, fluoroquinolones are recommended, but other drugs such as amoxicillin, ampicillin, or tetracyclines have been used. *E. coli*-associated granulomatous colitis (seen mostly in Boxer dogs), is treated with fluoroquinolones such as enrofloxacin (see Section Ulcerative colitis in dogs).

One of the drugs used for bacteria overgrowth in people is rifaximin (Xifaxan®). This is an FDA-approved oral antibiotic (200 or 550-mg tablet), that is minimally absorbed systemically (< 0.4%) and excreted in the feces. Because its activity is limited to the lumen of the intestine, it is used for treatment of traveler's diarrhea, hepatic encephalopathy, and irritable bowel syndrome. It has broad-spectrum activity against gram-negative bacteria, gram-positive bacteria, and anaerobes (including *Clostridium* species). Its use in animals has not been reported.

Tylosin-responsive diarrhea: Because some forms of chronic diarrhea in animals have been responsive to the antimicrobial tylosin (Tylan®), it has been characterized as "tylosin-responsive chronic diarrhea in dogs" (Westermarck et al., 2005a, 2005b). This disease, affecting both large and small bowel, is most likely caused by a bacterial pathogen, but the specific etiology has not been identified. *Campylobacter jejuni* and *Clostridium perfringens* have been isolated in some of the affected animals. Tylosin has been effective at improving clinical signs that occur with or without organisms being identified. When administered at 12 mg/kg/day (average dose) response was prompt. Some dogs respond within 24 hours; others respond within 3 days. These benefits appear to be unique to tylosin, because studies by the same investigators showed tylosin at a dose of 20 mg/kg/day was effective, but other drugs (metronidazole, trimethoprim–sulfonamides, doxycycline, or prednisolone) were not.

Sources of tylosin: The powdered form for livestock (Tylan®) has been mixed with the animal's food at a dosage of 40–80 mg/kg/day. One teaspoon contains approximately 3 grams (3,000 mg); therefore, one-quarter teaspoon contains 750 mg – enough for many dogs. Some animals may find the bitter taste unpleasant and refuse their food; therefore, the administration should be tested to identify the method of administration that is best tolerated. Tablets for small animals are available in other countries.

Metronidazole (Flagyl)-responsive diseases: Metronidazole is active against the protozoan, *Giardia*. It is for this use that metronidazole was first administered to treat diarrhea in small animals. Veterinarians discovered that metronidazole also was effective in patients that did not have giardiasis. Bowel inflammation may be caused by metronidazole-sensitive bacteria, which act as an attractant for inflammatory cells. The efficacy of metronidazole may be related to this antibacterial activity. Oral administration of metronidazole at 20 mg/kg to cats decreased the number of anaerobic and aerobic bacteria and altered the indigenous bacterial population (Johnston et al., 2000). There may be an immune-modulatory effect in the intestine (decreased cell-mediated response), but this has not been well characterized. Metronidazole (with and without combination with ampicillin) has been effective for histiocytic ulcerative colitis in dogs (Hostutler et al., 2004). Doses have been as high 60 mg/kg/day for 2 to 4 weeks, but these high doses carry a higher risk of adverse effects; 15 mg/kg twice daily is a safer dose to consider. The dose administered frequently to cats is one-quarter of a 250 mg tablet, two to three times daily (10 to 25 mg/kg). After a response is observed the frequency of administration should be decreased. *One must caution pet owners that when the tablet is broken, the exposed tablet may cause an unpleasant taste for cats.*

Adverse effects from metronidazole are primarily seen as CNS effects, and are discussed in more detail in Chapter 42 of this book. Tremors, seizures, and other CNS disturbances have been documented. CNS problems are caused by inhibition of GABA and are more likely with high doses.

Ronidazole: Ronidazole is from the same chemical group as metronidazole. Compared to metronidazole, it has greater in vitro activity against the protozoa organism *Tritrichomonas foetus*, which causes intestinal infections and diarrhea/lose stools in cats. Ronidazole is one of the few drugs associated with eradication (Gookin et al., 2006). Therefore, it is now the preferred treatment for intestinal infections in cats caused by the organism. It has been administered at a dose of 30 mg/kg once or twice daily for 14 days (once daily is preferred).

Although CNS reactions have been associated with ronidazole treatment in cats, these effects are usually associated with high doses. Because of the long half-life, it may be possible to administer this drug once daily and avoid adverse effects.

Other nitroimidizoles: Tinidazole (Tindamax) is used in people to treat intestinal protozoal infections. The use of tinidazole in animals has only been anecdotal.

***Campylobacter* enteritis:** *Campylobacter jejuni* is a gram-negative microaerophilic curved motile rod. Most infections in animals are self-limiting and not treated. However, the organism may be transmitted to people and this can be a public health concern. This organism is the most common cause of food-borne infectious diarrhea in people. The treatment is aimed primarily at maintaining fluid and electrolyte balance, but if antimicrobials are administered, a macrolide antibiotic is usually the first choice (including azithromycin). Other antimicrobials that may be considered are fluoroquinolones, clindamycin, tetracycline, or chloramphenicol.

Ulcerative colitis in dogs: In some dogs, particularly Boxer dogs, ulcerative colitis has been associated with *Escherichia coli* infection of the colonic mucosa (Mansfield et al., 2009). The invasive bacteria evoke production of inflammatory reactions and produce cytokines responsible for clinical signs. This leads to mucosal infiltration with large numbers of macrophages that induce an inflammatory reaction in the bowel, leading to ulcerative colitis and clinical signs. This differs from the experience with ulcerative colitis in people in which bacteria do not seem to play a role (Danese and Fiocchi, 2011). Successful treatment has been accomplished with oral administration of fluoroquinolones, which are highly active against *E. coli.* One of the regimens includes enrofloxacin at 7 mg/kg, once a day, for a duration of 9 weeks, but it is possible that a shorter course of treatment also would be effective.

Salmonella: The primary goal when treating *Salmonella* is to maintain fluid balance and prevent electrolyte losses. The patients should be isolated to prevent spreading of infection and strict containment procedures should be instituted in the hospital. Antibiotics are discouraged unless absolutely necessary for patients with *Salmonella* because antibiotic therapy may prolong shedding. In horses, if there is fever and neutropenia, intravenous gentamicin should be considered. Other drug choices include the following: fluoroquinolones, chloramphenicol or florfenicol (depending on the species), ampicillin or amoxicillin or amoxicillin–clavulanate, trimethoprim–sulfonamide, or a cephalosporin (the activity against *Salmonella* is highest with third-generation cephalosporins).

Antibiotic Treatment in Calves and Pigs

Use of antibiotics to treat diarrhea is common in young ruminants and pigs. A review of the relevant literature was summarized by Constable (2004). This author points out that the following antimicrobials are approved for treatment of calf diarrhea: amoxicillin, chlortetracycline, neomycin, oxytetracycline, streptomycin, sulfachloropyridazine, sulfamethazine, and tetracycline, *all* administered orally. Some of these drugs were approved many years ago – approximately 50 years ago in some cases – before evidence was required for approval. Of all these drugs listed, only amoxicillin has been shown to be effective in well-controlled studies. Other drugs may be effective, (fluoroquinolones, chloramphenicol, nitrofurazone) but it is a violation of federal regulations to administer these drug for this indication in calves. If bacteremia develops as a complication of bacterial diarrhea in calves, systemic therapy is needed. Drugs most often given systemically (IM, SC, or IV) for this indication include ceftiofur and ampicillin.

In baby pigs, there are oral formulations of aminoglycosides (e.g., gentamicin – *Garacin Pig Pump*) to be administered orally to baby pigs 1–3 days of age. Other forms of gentamicin are intended to be added to drinking water to treat diarrhea in weaned pigs or for swine dysentery (1–3 day treatment). Other aminoglycosides (e.g., neomycin and amikacin) also have been administered orally to pigs for diarrhea.

Antibiotic Treatment for Diarrhea in Foals

Diarrhea in foals is common. Normal nursing can maintain hydration in mild cases. But in more severe cases, fluid and electrolyte therapy is needed to maintain hydration. For infections caused by *Clostridium* spp., metronidazole has been used. For treatment of *Salmonella* (if indicated) aminoglycosides and/or cephalosporins (systemically) can be used, especially if systemic treatment is indicated with signs of sepsis. In horses 3–6 months of age, a disease characterized as *proliferative enteropathy* is caused by the organism *Lawsonia intracellularis*, so-named because it is an obligate intracellular pathogen. Affected animals show signs of lethargy, anorexia, depression, weight loss, colic, diarrhea, and hypoproteinemia. Treatment in horses has included erythromycin, azithromycin, chloramphenicol, clarithromycin, and doxycycline. Tetracyclines (doxycycline or oxytetracycline) are preferred. In pigs *Lawsonia intracellularis* also causes intestinal disease. The recommended treatment is a tetracycline and tylosin, added to feed.

Drugs for Treatment of Inflammatory Intestinal Diseases

Intestinal inflammatory diseases cover a wide range of etiologies grouped together because many have similar clinical signs. Among the diseases that may be included in this group are those caused by parasites, bacteria, food hypersensitivity, or immune-mediated diseases. Inflammatory bowel disease (IBD) may involve the small intestine or large intestine. There are similarities to intestinal diseases in people, which were reviewed by Hanauer (1996).

Inflammatory bowel disease (IBD) is a collective term that describes a group of disorders characterized by persistent or recurrent signs and histological evidence of inflammation that may involve the small or large intestine. Inflammation may be characterized by the cells infiltrating the mucosa (lymphocytic, plasmacytic, eosinophilic, neutrophilic). When there are bacterial causes, IBD may be the result of overexuberant inflammatory response to a bacteria invading the intestinal mucosa. In the large intestine, the disease is often referred to simply as colitis but also may be associated with many etiologies. Chronic colitis may be lymphocytic, plasmacytic (most common), eosinophilic, or granulomatous, which is associated with macrophages and inflammatory cells.

Initial therapy for inflammatory intestinal disease is aimed at managing the underlying cause. Therefore, antiparasitic therapy and dietary therapy are usually pursued initially. Antiparasitic drugs are discussed in Chapters 39–42. Dietary management and probiotics are also used for treatment. When these treatments have failed, or a specific etiology has not been identified, drug therapy consisting of antiinflammatory drugs is used, or combined with other treatments.

Sulfasalazine (Azulfidine®)

Sulfasalazine is used as an initial drug therapy for treatment of colitis. It is not as effective for inflammatory diseases of the small intestine. Sulfasalazine is a combination of sulfapyridine and 5-aminosalicylic acid (mesalamine) joined by an azo bond. The bond is broken by bacteria in the colon to release the two drugs (Figure 46.4). The sulfonamide component is absorbed into the circulation while the salicylic acid component remains active in the GI tract. Less than half of the salicylate component is absorbed systemically.

Clinical Use

Many veterinarians consider sulfasalazine to be the drug of choice for the initial treatment of ulcerative or idiopathic colitis, or plasmacytic–lymphocytic colitis after dietary therapy has been attempted. Clinical efficacy is attributed to a local antiinflammatory action from the salicylate component. There is also evidence for antilipooxygenase (LOX) activity, decreased IL-1, decreased prostaglandin synthesis, and oxygen radical scavenging activity.

Dosage recommendations start at 10–25 mg/kg q 8 h, PO, but have been as high as 40 mg/kg, two to three times per day. The dose is then reduced after there is an initial response to treatment.

Adverse Effects

The salicylate component is absorbed only minimally. The sulfonamide component is absorbed and can produce adverse effects in some animals, such as keratoconjunctivitis sicca in dogs (Morgan and Bachrach, 1982). This drug cannot be used in patients that have allergic reactions to sulfonamides.

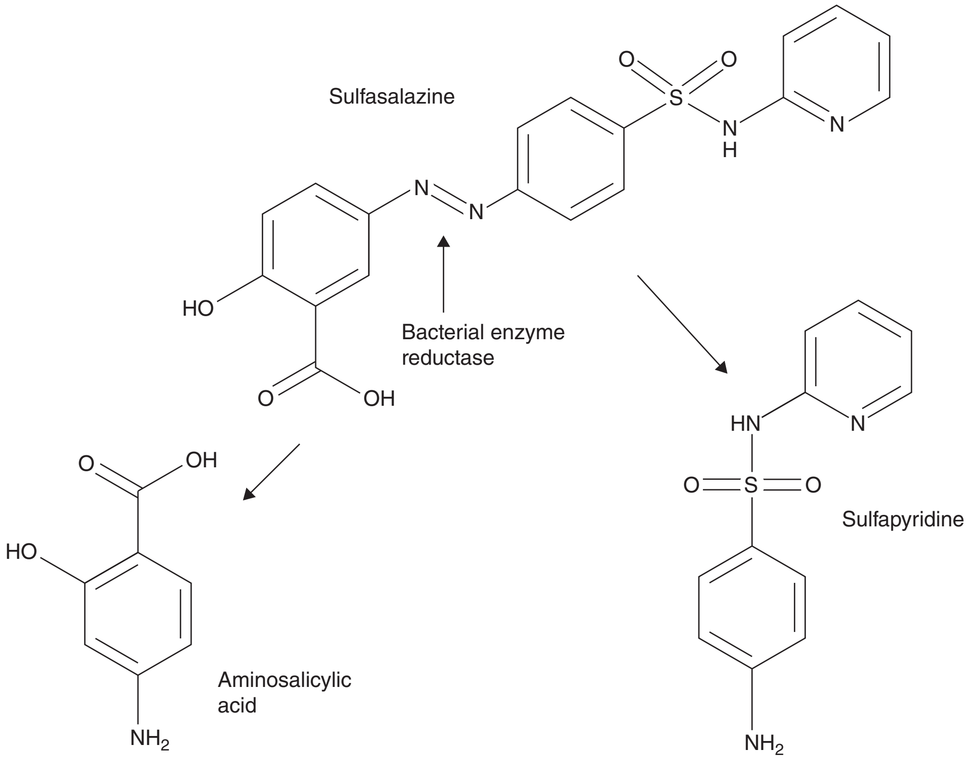

Figure 46.4 Sulfasalazine is a combination of sulfapyridine and 5-aminosalicylic acid (mesalamine) joined by an azo bond. The bond is broken by bacteria in the colon to release the two drugs. The sulfonamide component is absorbed, but the salicylic acid component remains active in the GI tract.

Other Sources of 5-Aminosalicylic Acid (Mesalamine)

In people, the adverse effects associated with sulfasalazine therapy are primarily due to the sulfonamide component; therefore, drugs have been formulated to contain mesalamine (5-aminosalicylic acid) without the sulfonamide. These formulations are listed in Table 46.8. Some formulations are designed to release the active drug at the distal portion of the intestine or colon because generally the pH is higher in these regions of the intestine.

Anticholinergic Drugs (Antimuscarinic Drugs)

As discussed previously, anticholinergic drugs (atropine-like drugs) inhibit smooth muscle and glandular secretions of the GI tract (also discussed in Chapter 8). Anticholinergic drugs occasionally have been used for the treatment of colitis. However, colitis is not ordinarily associated with hypermotility and the rationale of anticholinergic drugs should be questioned. Patients with a hypermotile or spastic bowel may have a limited benefit from these drugs.

Anticholinergic drugs also may have a role for reducing straining during the postoperative management of rectal and/or anal surgical procedures. A common drug for these indications is propantheline (Pro-Banthine®) at a dosage of 0.25 mg/kg, orally, two or three times daily.

Table 46.8 Mesalamine (5-aminosalicylic acid) formulations

Asacol®: Asacol is a tablet coated with an acrylic-based resin. The resin dissolves at a pH of 7.0 and is designed to release 5-aminosalicylic acid in the colon. It is available as a 400 mg tablet.

Mesasal®: Mesasal is a tablet coated with an acrylic-based resin that dissolves at a pH of >6.0. It is designed to release 5-aminosalicylic acid in the terminal ileum and colon. Approximately 35% of the salicylate is absorbed systemically. It is available in 250 and 500 mg tablets. The dose in people is 1 to 1.5 g/day.

Olsalazine sodium (Dipentum®): Olsalazine is a dimer of 2 molecules of 5-aminosalicylic acid linked by an azo bond. It is used in people who cannot tolerate sulfasalazine. Only 2% of the salicylate from this compound is absorbed systemically. It is available in 500 mg tablets. The most common adverse effect in people from this preparation has been a watery diarrhea.

Pentasa®: This formulation contains microgranules of mesalamine coated with ethyl cellulose, which releases 5-aminosalicylic acid into the small and large intestine gradually, regardless of pH.

Glucocorticoids

The effects of glucocorticoids for treating inflammatory bowel diseases are probably related to their antiinflammatory and immunosuppressive ability (Dillon, 1989).

It is believed that colitis is the result of autoantibodies and T-lymphocytes directed against the colonic epithelial cells. Corticosteroids, via their ability to suppress cytokine synthesis, leukocyte migration, and lymphocyte activation suppress the activity responsible for inflammatory bowel disease clinical signs. Glucocorticoids have been used when a biopsy suggests eosinophilic colitis or lymphocytic–plasmacytic colitis (Washabau and Holt, 2005; Leib et al., 1989). They are often employed when other forms of therapy (including dietary) have failed. The pharmacology of the corticosteroids is discussed in more detail in Chapter 29, and with immunosuppressive agents in Chapter 45.

Clinical Use and Dosages

The dose of prednisolone initially is in the immunosuppressive range of 2 to 4 mg/kg per day in divided doses for dogs and cats. Although there are no comparisons between prednisone and prednisolone, it is advised to use prednisolone. Prednisone is an inactive drug that relies on the conversion to the active drug prednisolone. If a local (topical) effect is desired from an oral dose, prednisolone is preferred over prednisone. Moreover, there is evidence in cats that some animals do not convert prednisone to the active drug.

After initial response is observed, the doses are tapered to approximately 1 mg/kg every other day if there is a positive treatment response. Prednisolone may be administered with azathioprine (see section Azathioprine). The magnitude of the dosage depends on the severity of the disease. Cats with severe inflammatory bowel disease have required prednisolone dosages as high as 4 mg/kg/day. Injections of corticosteroids (for example, methylprednisolone acetate DepoMedrol® at 20 mg/cat) for the control of inflammatory bowel disease has not been as successful as oral therapy. *Veterinarians should be aware that some animals develop diarrhea when treated with glucocorticoid therapy.*

Local Treatment with Budesonide (Entocort EC)

Budesonide is a locally acting corticosteroid. It has been used in people, but there also has been used in dogs and cats. Budesonide granules are contained in an ethylcellulose matrix that is coated with methacrylic acid polymer. This coating does not release the drug until the pH >5.5. In the proximal intestine the pH is low and it gradually increases in the distal intestine to attain a pH above 7. Therefore, the drug is not released until it reaches the distal GI tract. If a portion of the drug is absorbed, 80–90% is inactivated by first-pass metabolism. Therefore, systemic glucocorticoid effects are minimized. In humans it has been as effective as other drugs for treatment of Crohn's disease. It is available in a 3-mg capsule and is used in people at a dose of 9 mg/day.

Use of budesonide in animals: The experience with budesonide in dogs and cats has been limited, but provide some evidence of a beneficial effect. The intestinal pH is generally higher in small animals than people and a quicker release of the active drug is possible compared to people. Although systemic absorption is small, some may occur because there was a decreased response to ACTH after 30-day treatment to dogs at 3 mg/m^2. Other adverse effects were not observed (Tumulty et al., 2004).

Immunosuppressive Drugs

Immunosuppressive drugs also have been used to manage some forms of inflammatory bowel diseases. The most commonly used immunosuppressive drug for intestinal disease is azathioprine. The use of other drugs, such as cyclosporine, has been more limited.

Azathioprine

Azathioprine (discussed in Chapter 45) is metabolized to active metabolites that inhibit activated lymphocytes thereby suppressing lymphocyte response. Azathioprine (Imuran®) has been used at dosages of 2 mg/kg once daily and 40 mg/m^2 once daily in dogs with severe inflammatory bowel disease. The dose may be decreased to 0.5 mg/kg every other day if there is a positive initial response. Azathioprine has caused other GI problems during therapy (e.g., diarrhea during the initial therapy and liver injury with longer use); therefore, careful monitoring during treatment is recommended. Cats are more sensitive to the adverse effects than dogs and other drugs are usually administered to cats (e.g., chlorambucil, see Section Chlorambucil).

Chlorambucil

Chlorambucil, an alkylating agent, also is used in combination with prednisolone for treatment of chronic enteropathy and inflammatory bowel disease. The dose administered has been 4–6 mg/m^2, oral every 24 hours. If there is a positive response, the dose and frequency can be reduced. In one study, the combination of chlorambucil–prednisolone was more effective than the combination of azathioprine–prednisolone for treating chronic enteropathy and protein-losing enteropathy in dogs (Dandrieux et al., 2013).

N-3 Fatty Acids For Colitis

In people, omega-fatty acids (n-3 fatty acids) such as eicosapentanoic acid are beneficial for the treatment of colitis. These compounds are available in veterinary medicine in products such as DermCaps®, Pet-Derm®, and others for the purpose of treating pruritic skin disease. In studies performed in people with ulcerative colitis, supplementation for 4 months with omega fatty acids reduced leukotriene levels in the colon, and

Table 46.9 Use of laxatives and cathartics in veterinary medicine

Promotes the elimination of a soft-formed stool
Increases passage of intestinal contents to relieve an impaction (equine)
Cleanses bowel prior to radiography or endoscopy
Promotes the elimination of a toxin from the intestine
Softens stools following intestinal or anal surgery (decreases straining)

improved clinical signs. The clinical efficacy of these compounds for treating small animals with colitis has not been reported.

Cyclosporine

Cyclosporine (Neoral®, Atopic®) has been used to treat perianal fistulas, which are a component of ulcerative colitis (pharmacology of cyclosporine is discussed in Chapter 45). The treatment for this disease has been very successful. In addition, cyclosporine has been used to treat diarrhea caused by inflammatory bowel disease (Allenspach et al., 2006). At a dose of 5 mg/kg PO, q 24 h, ×10 weeks there was improvement in 78% of dogs. The success is attributed to the ability of cyclosporine to suppress T-cell mediated activity in the bowel, and to suppress inflammatory cytokines.

Laxatives and Cathartics

Laxatives and cathartics are drugs that increase the motility of bowel and change the character of the stool. Uses of these drugs are listed in Table 46.9. These drugs promote the elimination of soft-formed stool or increase fluid content of stool, which increases the bulk. Laxatives and cathartics can be divided into (i) bulk-forming laxatives, (ii) stool softeners, (iii) lubricants, (iv) saline hyperosmotic agents, and (v) stimulants. They also may be categorized by their onset of action: the osmotic (saline) laxatives act in 1–3 hours; the direct stimulants act in 6–8 hours; the bulk laxatives and surfactants act in 1–3 days.

Stimulant (Irritant) Laxatives

The exact mechanism of action for this group of laxatives is uncertain, but they appear to cause electrolyte loss by inhibiting Na^+/K^+-ATPase in the intestine and increasing electrolyte loss through intestinal tight junctions. These drugs are among the most rapidly acting of the laxatives, with action within 6–8 hours. They can have potent effects and excessive fluid and electrolyte loss can result from overdoses. Adverse effects can result from damage to enterocytes from chronic use and abuse. Some of the agents available include:

diphenylmethane: use is not documented in veterinary medicine;
phenolphthalein: this was once an ingredient found in over-the-counter laxative preparations such as ExLax®, but is no longer available (other natural ingredients are now used in ExLax®);
anthraquinone: these are glycoside derivatives found in bisacodyl (Dulcolax®), senna (Senokot®), and cascara sagrada (Nature's Remedy®).

Hyperosmotic Cathartics (Saline Cathartics)

The saline cathartics are nonabsorbed electrolytes that draw fluid into the bowel via osmosis. These are the most rapidly acting of the cathartics, with onset of action in 1–3 hours. The fluid content of the stool increases, which causes intestinal distention and promotes increased normal peristalsis. These have been some of the most commonly used cathartics in veterinary medicine. Most often they are dissolved in an aqueous solution (water) and administered via gastric gavage (stomach tube). These drugs have been used to prepare animals for an endoscopic procedure (*bowel cleansing)* or for cathartic treatment of poisoning. They are relatively safe drugs, but overdoses can cause fluid loss in a patient. Examples of these drugs are shown in Table 46.10.

Hydrophilic Colloids ("Bulk Laxatives")

These agents are mostly natural products and dietary plant fibers. They are composed of nonabsorbed synthetic or natural polysaccharide and cellulose derivatives. These fibers are resistant to digestion and attract water into the intestine. By imbibing water, they increase the

Table 46.10 Saline cathartics

Sodium sulfate **(Glauber's salt)**
Magnesium salts such as magnesium sulfate (Epsom Salts), magnesium hydroxide (Milk of Magnesia), and magnesium citrate (Citro-Mag®)
Nonabsorbed sugars: Lactulose, Mannitol, Polyethylene glycol (PEG)
Lactulose also is used to treat hepatic encephalopathy. Its action lowers the bowel pH and decreases ammonia absorption by converting to NH^{+3}. The ionized form of the compound is less absorbed systemically.
Combination products: Golytely is a strong osmotic cathartic that is a combination of sodium sulfate, potassium chloride, sodium chloride, sodium bicarbonate, and polyethylene glycol (PEG 3350). The dose is 250 ml to one liter per animal (depending on the size) and has a rapid onset of action. The most common use of this product is for bowel cleansing prior to an endoscopic procedure.

Table 46.11 Bulk laxatives

Carboxymethylcellulose
Methylcellulose
Psyllium (Metamucil) powder
Prunes and wheat bran and other attempts to increase “fiber” in the diet
For cats, canned pumpkin is a source of fiber that most cats eat readily

mass of nondigestible material in bowel and motility is increased through the stimulation of mechanoreceptors. They are more slowly acting than other drugs with an onset of action of 24 hours, or longer. They are relatively safe drugs with few side effects. Examples are included in Table 46.11.

Lubricant Laxatives (Mineral Oil and Liquid Petrolatum)

The lubricants act by coating the surface of the stool with a water-immiscible film and increasing the water content of the stool. They also produce a lubricant action to ease passage of the stool. Lubricant laxatives usually contain mineral oil (liquid petrolatum) or white petrolatum. Many of the nonprescription products contain white petrolatum (Vaseline®) as their ingredient. Administration of glycerin also has been used as a lubricant laxative. The lubricant laxatives are relatively safe because they are absorbed from the GI tract only to a small extent. The worst adverse effect is that they may possibly elicit a foreign body reaction. Chronic use may decrease the intestinal absorption of fat-soluble vitamins.

Use in large animals: These drugs, particularly mineral oil, are popular for several nonspecific GI problems in horses and cattle.

Use in small animals: Products such as Laxatone®, Felaxin®, and Kat-a-Lax® are promoted for increasing the passage of trichobezoars (hair balls) in cats. These products contain white petrolatum (Vaseline) as their active ingredient with additional flavorings.

Stool Softeners (Surfactants)

These drugs act to decrease surface tension and allow more water to accumulate in the stool. Their onset of action is usually 24–48 hours. They are relatively safe, except cramping pains are reported in people if they ingest high dosages.

Docusates: Dioctyl sodium sulfosuccinate (DSS, Colace®) and dioctyl calcium sulfosuccinate (Surfak®, Doxidan®). Also known as docusate sodium and docusate calcium, these compounds are used when there is a need to soften the stool. Efficacy in animals has not been tested.

Bile acids: Bile acids include dehydrocholic acid and ursodeoxycholic acid (Ursodiol, Actigall). Bile acids can produce both a choleretic (increase bile flow) and laxative effect. Ursodeoxycholic acid is better known by most veterinarians for other uses. Although listed with the laxatives, most of the use of ursodeoxycholic acid (Ursodiol®), in veterinary medicine is for the management of liver disease in animals, rather than for its laxative action. Ursodeoxycholic acid has an important effect in slowing progression, or improving, liver disease (Lindor, 2007). Because ursodiol is a water-soluble bile acid, administration may alter the circulating pool of bile acids to replace some of the more toxic, hydrophobic bile acids from accumulating in animals with liver disease.

Castor oil: Castor oil is hydrolyzed in the bowel to release ricinoleic acid, which causes an increased secretion of water in the small intestine.

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47

Dermatopharmacology: Drugs Acting Locally on the Skin

Jim E. Riviere and Rosanna Marsella

A large number of cases seen in the everyday practice of small- and large-animal veterinary medicine involve lesions of the skin or its appendages. This chapter reviews the salient features of the general anatomy, histology, and biochemistry of skin relevant to the treatment of dermatological disease; acquaints the practitioner with the features of percutaneous drug absorption relevant to the treatment of skin disease; and discusses the categories of pharmacological preparations available on the veterinary market to treat skin diseases in domestic animals. The specific drugs used to treat skin diseases are well covered in other chapters of this text. Chapters that should be consulted for application to skin disease include those on antimicrobials, analgesics, and antiinflammatory drugs; antipruretics and antihistamines; glucocorticoids; immunosuppressives (cyclosporine); and ectoparasiticides.

Only in the last few decades have scientists begun to understand how the skin functions in normal and diseased states, to understand the skin's barrier function in terms of water loss and drug delivery, and that attempts have been made to improve the delivery of pharmaceutical agents through the skin by temporarily adjusting that barrier to deliver the drug. In order to successfully minimize the skin's ability to block the absorption of drugs to treat dermatological disease in domestic animals, it is imperative to understand the normal functional anatomy and biochemistry of the skin. In veterinary medicine, transdermal delivery is widely employed when pesticides are applied monthly to a single area of skin for the control of fleas and ticks over the entire body. Transdermal fentanyl patches are also widely used for postsurgical analgesia. This topical port of drug delivery will see increased use for other therapeutic indications. However, most drugs applied to the skin are not intended to be absorbed systemically but are used to elicit a local therapeutic effect in the treated skin.

The characteristic that separates dermatological therapy from other components of veterinary pharmacology is this use of topical dosage formulations to treat surface conditions or to target the underlying skin. The drugs are not different from those used in other diseases, but they are different in the vehicles employed and mode of administration. A full understanding of the biological factors that modulate absorption and of the pharmaceutical composition of dermatological formulations (e.g., vehicles) is essential to a proper understanding of dermatopharmacology. The same principles also apply to the absorption of topical transdermal preparations such as pour-on ectoparasiticides and analgesics such as transdermal fentanyl gels or patches. It must be stressed that the drugs incorporated into these formulations are the same as those used to treat diseases of other systems and thus, their descriptions will not be repeated in this chapter.

Anatomy and Histology

The skin is the largest organ of the body, with the integument accounting for 24% of the overall body weight in the puppy and 12% in the adult dog (Pavletic, 1991). The skin has the function of protecting the internal organs of the body from extremes in temperature fluctuations, allergens, pollutants, toxic chemicals, and organisms such as bacteria, fungi, parasites, and viruses found ubiquitously in the environment.

The skin of domestic animals is quite similar in gross and histological morphology across species lines and is usually thickest over the head, dorsum of the neck, back, and sacrum and on the plantar and palmar surfaces of the feet. It is thinner on the ventral abdomen, the medial surfaces of the limbs, and the inner pinnae, and thinnest over the scrotum of male animals and the earlobe of the human. Perforating the skin are several types of appendages (depending on the species), such as hair follicles, sebaceous and sweat glands, spines, quills, scales, spurs, horns, claws, nails, and hooves (Montagna, 1967). The specific anatomy of skin and hair has been reviewed extensively elsewhere (Monteiro-Riviere, 2006; Blackburn, 1965; Lloyd et al., 1979a, 1979b; Sar and Calhoun, 1966; Kozlowski and Calhoun, 1969; Strickland

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and Calhoun, 1963; Talukdar et al., 1972; Pavletic, 1991; Montagna, 1967; Amakiri, 1973).

The Epidermis

On the histological level, the skin can be divided into two distinct units: the epidermis and the dermis. The epidermis consists of stratified squamous keratinized epithelium that undergoes a programmed proliferation and differentiation that will eventually result in the formation of the major barrier to drug penetration: the stratum corneum. Two primary cell types exist in the epidermis: those of keratinocyte origin and those of nonkeratinocyte origin.

Five distinct layers of keratinocytes can be present in the epidermis, as shown in Figure 47.1. Listed from the deepest layer of the epidermis to the most superficial, they are (i) stratum basale (basal layer), (ii) stratum spinosum (prickle layer), (iii) stratum granulosum (granular layer), (iv) stratum lucidum (clear layer), and (v) the stratum corneum (horny layer). Each cell layer has its point of origin at the stratum basale. The stratum basale is a single layer of cuboidal or columnar cells that rest on the basal lamina. These cells are attached to the basal lamina by hemidesmasomes, and to each other and to the cells of the stratum spinosum by desmasomes. The stratum basale cells continuously divide, with some remaining as basal cells and others beginning to move more superficially and mature by changing their intracellular content through the process called *keratinization*. This viable and dividing epidermal cell layer is the target for transformation into cancerous cells.

The next more superficial layer next to the stratum basale, the stratum spinosum, is composed of irregularly shaped polyhedral cells that make up much of the bulk thickness of the epidermis. The next layer composes the stratum granulosum, which consists of several layers of cells that begin to flatten horizontally. Of primary interest are the lamellated granules within these cells, which contain polar phospholipids, such as glycosphingolipids and free steroids, and numerous hydrolytic enzymes, including acid phosphatase, proteases, lipases,

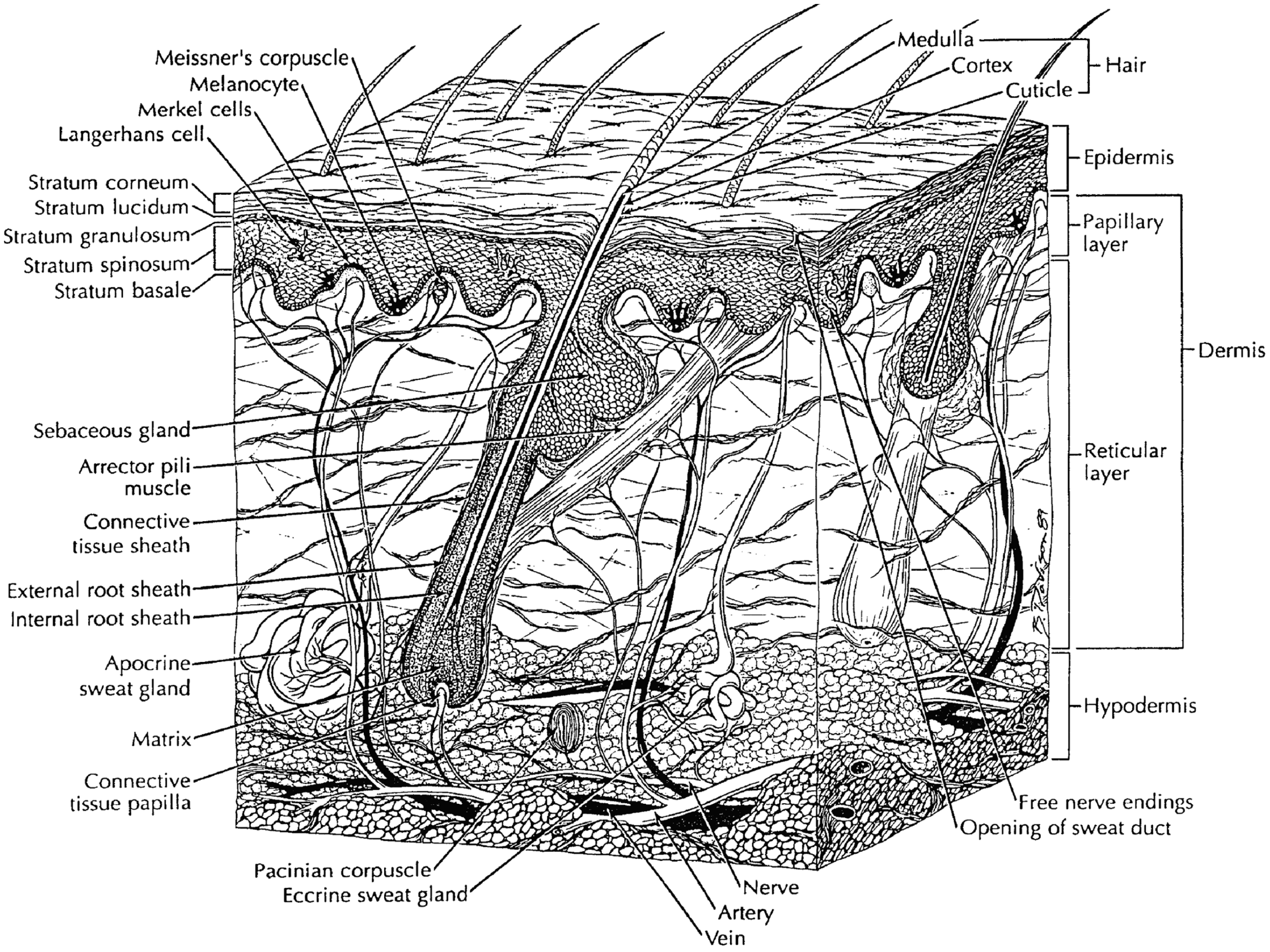

Figure 47.1 A schematic view of the epidermis and dermis of the skin. Source: Monteiro-Riviere, 1991. Reproduced with permission of Taylor & Francis Group LLC.

and glycosidases. As these intracellular products accumulate, these cells will exocytose their intracellular products and fill in the intercellular spaces, eventually forming the extracellular lipid matrix of the stratum corneum that forms the penetration barrier of the stratum corneum. As these epidermal cells continue their migration, they form the stratum lucidum, a translucent line of cells found only in areas having very thick skin, such as plantar and palmar surfaces (footpads) and the planum nasale. These cells are translucent because both nuclei and cytoplasmic organelles are missing (Monteiro-Riviere, 1991, 2006; Idsen, 1975; Montagna, 1967).

The stratum corneum is the final and most superficial layer of the epidermis and is its most important when considering the feasibility of topical drug therapy since it is the primary barrier to percutaneous absorption. In addition to the barrier function for xenobiotics trying to enter the body from the environment, the stratum corneum also provides a barrier to insensible water loss, an evolutionary adaptation that allows terrestrial animals to exist in a nonaquatic environment. In fact, most veterinary dermatological vehicles are targeted at this action. This layer consists of several dead layers of cells, organized into vertical columns in a tetrakaidecahedral (14-sided) configuration, the thickness of which varies depending on location (Monteiro-Riviere, 1991). This particular cell shape provides a minimal surface-to-volume ratio and also minimizes systemic water loss through the skin (transepidermal water loss). Each cell is ingrained in the lipid matrix produced by the lamellated granules when the cells were still in the stratum granulosum layer. These dead cells are also surrounded by a thick plasma membrane with a submembranous layer of involucrin, also produced earlier in development. With intracellular and intercellular barriers firmly in place, the stratum corneum has the ability to constrain the passage of unwanted chemicals and toxins from the environment. Unfortunately, the stratum corneum does not discriminate between these unwanted substances and the pharmaceuticals the veterinarian may wish to penetrate the skin for topical drug therapy for the treatment of dermatological disease.

Melanocytes are cells located in the basal layer of the epidermis and contain dark cytoplasmic granules called melanosomes. These cells impart color to the skin, the color and intensity determined by the number, size, distribution, and degree of melanization of the melanosomes. Merkel cells are also located in the basal region of the epidermis and are thought to function as slow-adapting mechanoreceptors for touch. Langerhans cells are located in the stratum spinosum but can also be present in dermal lymph vessels, lymph nodes, and dermis. The Langerhans cells' primary function is to present antigen to lymphocytes; they may also be the initial receptors for cutaneous immune responses.

The Dermis

The dermis is composed of connective tissue, consisting of collagen, elastin, and reticular fibers dispersed in an amorphous ground substance, and can be divided into two rather poorly demarcated areas. The papillary layer consists of loose connective tissue and connects the epidermis (stratum basale/basal lamina) to the deeper reticular layer of the dermis. The reticular layer consists of dense connective tissue connected to the hypodermis, which is composed mostly of fat.

Dispersed throughout both layers of the dermis is a network of arterial and venous blood vessels needed to nourish the cells of the epidermis and dermis as well as take part in the last stages of the percutaneous absorption of compounds. Lymph vessels, nerves, apocrine and eccrine sweat glands, sebaceous glands, Pacinian (pressoreceptor), Meissner's (touch receptor), and Ruffini (mechanical receptor) corpuscles, hair follicles, and smooth muscles (arrector pili) are the other major structures found in the dermis. Two types of arteries, musculocutaneous and direct cutaneous, supply the needed nutrients to the epidermis. Direct cutaneous arteries run parallel to the skin, directly supplying the skin with blood, while musculocutaneous arteries supply both the skin and underlying musculature and run perpendicular to the skin. Cutaneous blood supply by each type of artery varies with species and location. Cutaneous blood flow rates may be one of the factors affecting the passive percutaneous absorption of chemicals. Table 47.1 clearly demonstrates this by comparing laser Doppler cutaneous blood flow parameters in nine species of domestic animals (Monteiro-Riviere et al., 1990).

Species Differences

As a general rule, skin structure and function are similar across species lines. However, some minor differences are apparent.

Avian integument possesses the most profound differences in skin morphology from other domestic species. The four layers of the epidermis are, from the deepest to the most superficial, the stratum basale, the stratum intermedium (stratum spinosum), the stratum transitivum (stratum granulosum), and the stratum corneum (stratum germinativum). Unlike mammals, avians possess no skin glands (Monteiro-Riviere, 2006). The skin of aquatic mammals has a very thick stratum corneum resembling parakeratosis and there is no stratum granulosum (Montagna, 1967).

Pig skin is similar histologically to human skin (Monteiro-Riviere and Stromberg, 1985) and has been used experimentally to reliably predict the percutaneous absorption of chemicals in humans (Riviere, 2006).

Table 47.1 Cutaneous blood flow measurements in nine species of domestic animals. Source: Adapted from Monteiro-Riviere et al., 1990.

	Buttocks	Pinnae	Humeroscapular Joint	Thoracolumbar Joint	Ventral Species Abdomen
Feline	1.82 ± 0.59	6.46 ± 2.30	1.86 ± 0.70	2.39 ± 0.35	6.19 ± 0.94
Bovine	6.03 ± 1.84	6.98 ± 2.19	5.51 ± 2.32	5.49 ± 1.49	10.5 ± 2.13
Canine	2.21 ± 0.67	5.21 ± 1.53	5.52 ± 1.31	1.94 ± 0.27	8.78 ± 1.40
Equine	3.16 ± 1.22	NA	6.76 ± 1.49	2.99 ± 0.86	8.90 ± 1.46
Primate	3.12 ± 0.58	20.9 ± 5.37	8.49 ± 3.28	2.40 ± 0.82	3.58 ± 0.41
Mouse	3.88 ± 0.92	1.41 ± 0.48	10.1 ± 3.51	20.6 ± 4.69	36.9 ± 8.14
Porcine	3.08 ± 0.48	11.7 ± 3.02	6.75 ± 2.09	2.97 ± 0.56	10.7 ± 2.14
Rabbit	3.55 ± 0.93	8.38 ± 1.53	5.38 ± 1.06	5.46 ± 0.94	17.3 ± 6.31
Rat	4.20 ± 1.05	9.13 ± 4.97	6.22 ± 1.47	9.56 ± 2.17	11.4 ± 5.53

Values are ml/min/100 g tissue, ± standard error of the mean.
NA, data not available.

With respect to cutaneous circulation, musculocutaneous arteries are the primary vascular supply to the skin of humans, apes, and swine. Loose-skinned animals (canines and felines) lack musculocutaneous arteries; all vessels involved in cutaneous circulation travel parallel to the skin (Pavletic, 1991). Studies of piroxicam (a nonsteroidal antiinflammatory drug) in pigs suggest that the type of cutaneous circulation may affect the local tissue concentrations of topically applied drug (Monteiro-Riviere et al., 1993). Little work has been done directly comparing the absorption of drugs and topical pesticides across veterinary species (Riviere and Papich, 2001).

Biochemistry

Energy Production and Utilization

The skin, specifically the epidermis, is mostly an anaerobic organ. The absence of capillaries directly feeding oxygen to the epidermal cells makes the epidermal cells relatively oxygen poor in the normal state when compared to other tissues with a more direct blood supply. Due to this low oxygen tension, the epidermis produces 70–80% of its total energy requirements (adenosine triphosphate) through the anaerobic metabolic pathway (glycolysis), with lactic acid being the end product of glucose utilization. The epidermal cells, the most active of which are those in the single layer of the stratum basale, are the primary cells involved in this energy production, with the lactic acid end product passively diffusing into the dermis and then into the blood vasculature, eventually recycled by the liver back into glucose. Although the glycolytic pathway produces most of the energy requirements of the epidermis, other energy pathways (tricarboxylic acid cycle and pentose phosphate shunt) are also utilized to lesser degrees in some phases of epidermal growth (Freinkel, 1983).

Some topically applied drugs may have an effect on energy production pathways within the epidermis. One study (Spoo et al., 1993) showed that in vitro weanling porcine skin flaps had large increases in epidermal cell glucose utilization when the skin was dosed with benzoyl peroxide. In addition to drugs, vehicles (discussed in more detail in Section Topical Vehicles) may also have similar effects.

Drug Biotransformation

The stratum corneum is the primary line of defense to prevent percutaneous absorption of drugs. However, any drug passing through the stratum corneum may face a metabolic, rather than a physical, barrier. The skin has a remarkable ability to metabolize xenobiotics. These metabolic reactions consist mainly of oxidation, reduction, hydrolysis, and the phase I and II conjugation reactions. Enzymes present within the extracellular lipid matrix include acid lipase, phospholipase A, sphingomyelinase, glycosidases, acid phosphatases, cathepsins, and carboxypeptidases (Elias, 1992). Some of these reactions are major pathways for the metabolism of topical steroids as well as other drugs such as norepinephrine, benzoyl peroxide, and benzo(a)pyrene.

Skin has been shown to metabolize organophosphate parasiticides. When parathion is topically applied to the skin and penetration through the stratum corneum occurs, it undergoes significant metabolism within the epidermis to the bioactive metabolite paraoxon and/or *p*-nitro phenol, both of which will enter the systemic circulation to possibly affect other organ systems (Riviere and Chang, 1992). Additional studies have demonstrated the metabolic capabilities of skin when topically exposed to caffeine, testosterone, butylated hydroxytoluene, salicylic acid, norepinephrine, benzo(a)pyrene, and benzoyl peroxide – to name only a few – in many species of laboratory animals, as well as in human skin. Benzoyl peroxide, a popular veterinary drug used as a keratolytic and degreasing agent, is metabolized almost 100% to benzoic acid within the epidermis. In addition, the cytochrome P450 enzyme system, most commonly associated with the liver, is present and is inducible in skin (depending on the compound that was applied topically) and is the pathway that is responsible for the conversion of parathion into paraoxon in porcine skin (Riviere and Chang, 1992; Mukhtar, 1992; Bashir and Maibach, 2005). Glutathione-*S*-transferase, aryl hydrocarbon hydroxylase, and 7-ethoxycoumarin have also been demonstrated to exist in rat and mice epidermal cell homogenates (Raza et al., 1992). These studies all show that the skin has formidable metabolic functions as well as the traditional barrier functions.

Lipid Metabolism

In addition to its metabolic activities, skin also has a marked ability to synthesize lipid, which is used to construct the extracellular epidermal barrier. Epidermal cells manufacture a variety of neutral lipids, ceramides, glycosylceramides, gangliosides, sterol esters, fatty acids, alkanes, and phospholipids, which are largely found in the extracellular barrier of the stratum corneum. Some of these lipids tend to be site specific, with phospholipids and sterols residing mostly in the basal cell layers and some sterols and neutral lipids occupying the upper regions of the epidermis, mainly the stratum corneum. The effects on percutaneous absorption by the intercellular lipid structure have been discussed in greater detail elsewhere (Wertz, 1992; Potts and Francoeur, 1992; Hadgraft et al., 1992; Swartzendruber, 1992; Elias and Feingold, 1992; Monteiro-Riviere et al., 2001). It is now accepted that this complex intercellular lipid matrix provides the primary barrier to drug penetration. It is important to realize that in some skin disease states, the overall production of lipids by the epidermal cells may be altered due to altered intracellular metabolism, subsequently followed by some alterations in the percutaneous absorption patterns of many drugs. Alterations in the epidermal cells' plasma membrane (primarily composed of lipid) – whether due directly to a drug(s), by the vehicle a drug was delivered in, or due to a disease process – may incite inflammatory reactions due to the release of inflammatory mediators (eicosanoids) from the epidermal cells or from the underlying cutaneous vasculature.

Eicosanoids are a group of biologically active compounds derived enzymatically from eicosatetraenoic acid (arachidonic acid). Prostaglandins, leukotrienes, thromboxanes, and hydroxyeicosatetraenoic acids can be produced in minute quantities from arachidonic acid and have powerful proinflammatory effects on skin and the surrounding tissues. Eicosanoids are found in nearly every tissue in the mammalian body (Spannhake et al., 1981; Dunn and Hood, 1977; Goldyne, 1986), with some cells specializing in a certain type of eicosanoid. Skin produces several prostaglandins, including PGE_2, $PGF_{2\alpha}$, and PGI_2, as well as the lipoxygenase product leukotriene B_4.

PGE_2 induces vasodilation by elevating cyclic adenosine monophosphate in vascular smooth muscle cells, while $PGF_{2\alpha}$ causes vasoconstriction of the cutaneous vasculature by elevating cyclic guanosine monophosphate levels in vascular smooth muscle cells. In contrast to PGE_2, increased $PGF_{2\alpha}$ concentrations in skin enhance the leukocytes' response to chemotactic stimuli. $PGF_{2\alpha}$ has many effects on organs other than the skin, most notably in the reproductive tract of both humans and domestic animals. PGI_2 is a potent vasodilator produced mainly by vascular endothelial cells; it also inhibits vascular platelet aggregation. PGI_2 can be released from skin in response to some vehicles – namely, alcohols such as methanol, ethanol, and 2-propanol (Landolfi and Steiner, 1984; Karanian et al., 1985). Cutaneous vasodilation has been observed in humans orally consuming alcohol (ethanol), presumably due to release of PGI_2 from vascular endothelial cells in contact with the alcohol-containing blood (Landolfi and Steiner, 1984).

Leukotriene B_4 (LTB_4) is an inflammatory mediator produced from arachidonic acid via 5-lipoxygenase enzyme activity (Ford-Hutchinson, 1985). As with $PGF_{2\alpha}$, LTB_4 is a potent chemotactic agent (Paulissen et al., 1990; Van de Kerkhof et al., 1991) and may also be a potent vasodilator and cause significant increases in vascular permeability when coadministered with PGE_2 (Ford-Hutchinson, 1985). Although LTB_4 is most commonly produced by leukocytes, skin contains some inherent 5-lipoxygenase activity as determined from studies using stimulated human keratinocytes in culture as well as human and murine epidermal homogenates (Ruzicka, 1990). These inflammatory mediator-induced changes in cutaneous blood flow may impact on the absorption and cutaneous distribution of topically applied drugs.

Protein Metabolism

The primary protein product of the skin is keratin, which is the main intracellular component of the stratum corneum. In tandem with extracellular lipid, keratin forms the major component of the epidermal barrier. Keratin primarily consists of cystine, serine, glutamic acid, arginine, aspartic acid, and glycine amino acid residues. Of significance within the keratin molecule structure is the intrachain cystine disulfide bridging that further strengthens the overall keratin macrostructure. Keratin is the major protein foundation of hair (Monteiro-Riviere, 1991, 2006).

Principles of Percutaneous Absorption: Skin Permeability

Prior to the 20th century, many scientists believed that skin was an impermeable barrier to all substances, except possibly gases. The science of dermatology has since advanced to reveal that many substances can enter the systemic circulation via the skin. Four pathways by which substances can gain this access to the rest of the body have been postulated and are schematically shown in Figure 47.2.

The percutaneous absorption of drugs is important from three perspectives. Veterinary clinicians should be concerned about how much of a drug actually penetrates the skin to achieve a pharmacological effect in treating diseases of the skin. This can be a function of several factors including vehicle selection, hydration state of the stratum corneum, partition coefficient of the drug from vehicle into the stratum corneum lipids, the integrity of the stratum corneum, and cutaneous blood flow. The same endpoints, but in an opposite direction, also impact the efficacy and duration of effect for topical transdermal products. The final need for knowing the extent of percutaneous absorption lies in the field of public health and food consumption. Many compounds used in food animals are applied to and absorbed through the skin and enter the systemic circulation. Many of these compounds are stored in edible tissues (fat, muscle) for long periods of time, causing concern about residues in these tissues (Baynes et al., 1997; Riviere, 1992).

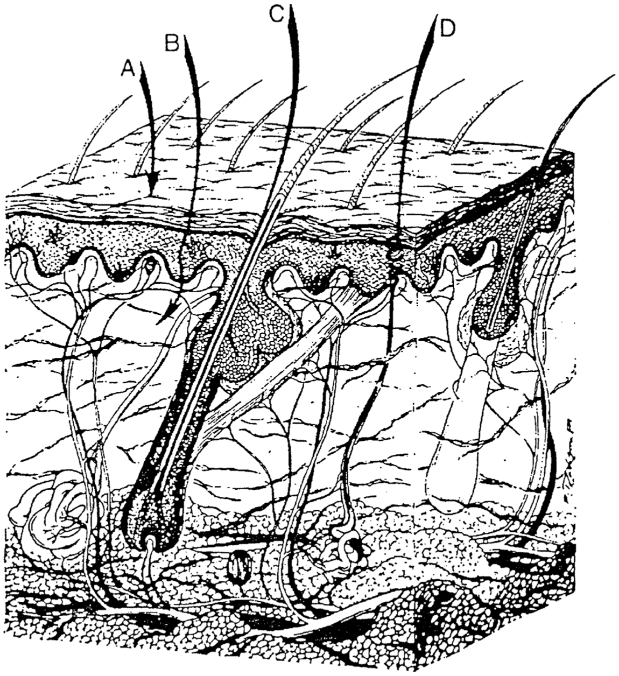

Figure 47.2 A schematic view of the skin and the four routes of drug penetration. Drugs can penetrate through the stratum corneocytes (A), between the stratum corneocytes (B), transfollicularly via any hair follicle (C), or via an eccrine sweat gland or sebaceous gland (D).

The stratum corneum is accepted to be the major barrier, but not the only barrier, to percutaneous penetration in most species of animals. Passive diffusion is defined here as the movement of matter from one space to another space by random molecular motion. No known active transport processes are used by stratum corneum to modulate percutaneous absorption. To better understand how substances diffuse through the stratum corneum, it is convenient to think of the stratum corneum as a wall in a "brick and mortar" configuration (Elias, 1983), with the bricks representing the stratum corneocytes and the mortar representing the lipid matrix around them, as shown in Figure 47.3. In this context, permeation through the stratum corneum might occur via two routes. The first involves the drug traversing through the stratum corneocytes ("bricks") and extracellular lipid matrix ("mortar"), through the cells of the deeper epidermal cells, and then into the systemic circulation. The second route involves the chemical maneuvering its way through the stratum corneum via the intercellular lipid matrix only. It is generally accepted that the primary route of penetration is via the intercellular pathway. Because of the structure of the stratum corneum and the metabolic capabilities of the lower epidermal cells, the veterinarian must be aware that only a small percentage of the drug that is topically applied is going to actually penetrate the stratum corneum. This small fraction of the total dose will need to be able to affect the disease process occurring in the underlying layers of the epidermis.

The molecular structure of the intercellular lipid matrix throughout the epidermis is in a liquid crystalline configuration, consisting of fatty acids, ceramides, triglycerides, sterols, sterol esters, cholesterol sulfate, and miscellaneous alkanes. As epidermal cells migrate superficially and transform into the cells of the stratum corneum, the lipid content surrounding these cells changes from polar to more neutral in nature. Specifically, phospholipids and triglycerides tend to decrease while fatty acids, cholesterol, cholesterol sulfate, ceramides, and sphingolipids increase as the epidermal cells differentiate (Elias, 1992). Enzymes are also present within this matrix of lipid. These lipids organize themselves into a lipid bilayer structure, with the hydrophobic ends of the molecules orienting themselves with other hydrophobic ends, and the hydrophilic ends orienting themselves in a similar fashion. More than one lipid bilayer can be formed within the intercellular matrix, leading to the formation of hydrophilic and hydrophobic channels, as illustrated in Figure 47.4. One might assume that this complex lipid barrier would result in very low absorption profiles for hydrophilic (water soluble) compounds since these molecules would

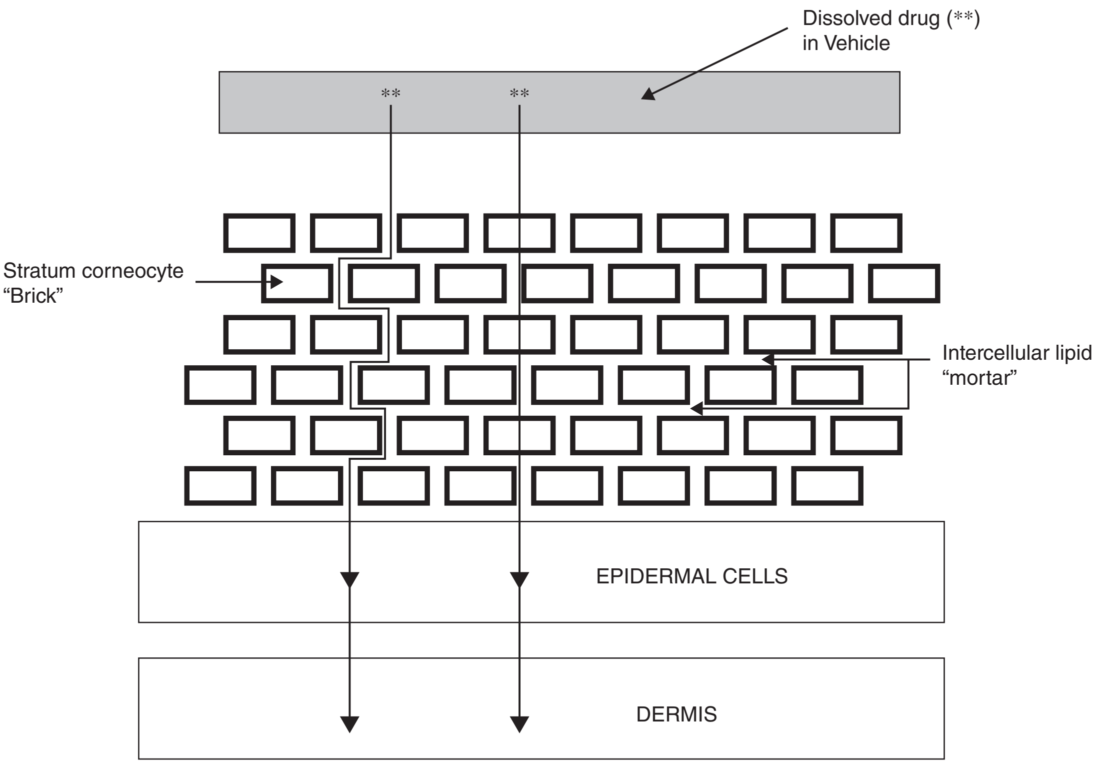

Figure 47.3 Simplistic "brick and mortar" model of the skin. Drugs can penetrate by traveling around the stratum corneocytes through the lipid matrix, or drugs can pass through each corneocyte via the lipid matrix.

have difficulty crossing a hydrophobic barrier, but some studies have shown that many hydrophilic compounds penetrate the epidermis in much higher quantities than predicted, implying that there are other mechanisms whereby hydrophilic compounds permeate the stratum corneum. The primary mechanism by which this is accomplished is for hydrophilic molecules to penetrate through fluctuations, or "kinks," in the lipid alkyl chains (Potts and Francoeur, 1992; Potts et al., 1992). Further, the permeation of hydrophilic molecules (such as water,

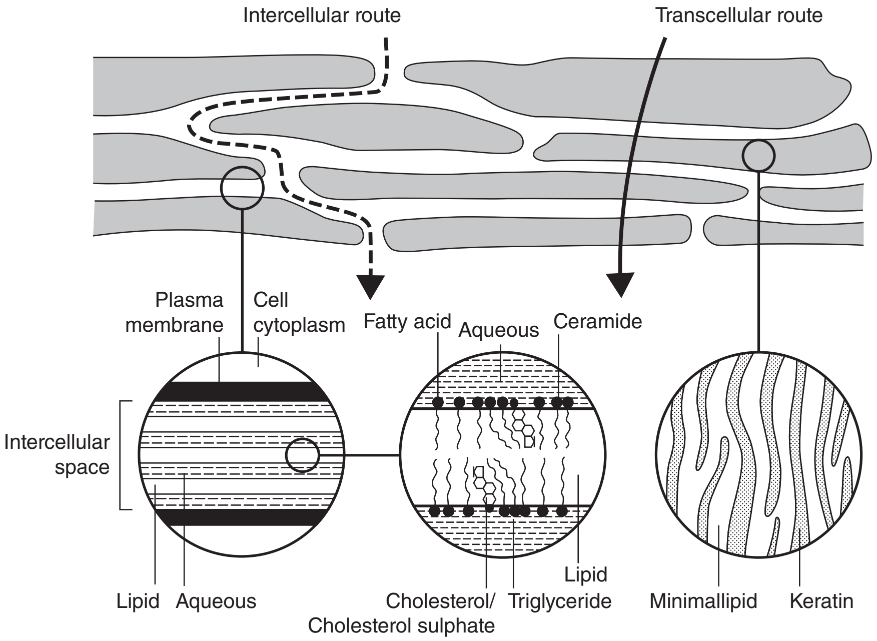

Figure 47.4 The lipid bilayer model of the stratum corneum.

methanol, ethanol, etc.) through the lipid matrix is due to their small size and molecular weight, which aid them in traveling through the lipid matrix via passages established by the lipid alkyl chains. This view is supported by the observation that larger hydrophilic molecules have lower percutaneous absorptions than their smaller molecular weight counterparts. Alternatively, penetration may also occur through the aqueous channels formed between the polar head groups of the lipid layers.

More recently, the role of tight junction in the barrier function of the skin and permeability to substances has been discovered. Tight junctions are present in the SG and are composed of a number of proteins that selectively and dynamically regulate the penetration of substances through epithelia, including the epidermis. Tight junctions are actively remodeled to allow the migration of keratinocytes toward the surface without compromising the barrier function of the skin (Matsui and Amagai, 2015).

Chemicals may also permeate the skin using the skin appendageal route, mostly by way of hair follicles and sweat ducts. The importance of the transappendageal route on percutaneous absorption is controversial, but some studies indicate that the importance of this route tends to be species specific (Pitman and Rostas, 1981). Generally, those animals possessing a sparse number of hair follicles per area of skin (humans, pigs) are considered to have little, if any, absorption of topically applied compounds via this route when compared to those animals having a high density of hair follicles (cattle, sheep). In these latter animals, the stratum corneum barrier is just as impermeable to drugs as that of their less-haired counterparts, but the drug permeates the skin barrier via the hair follicles, sweat ducts, or other openings in the stratum corneum, increasing the percutaneous absorption of the chemical. It has been demonstrated that transappendageal absorption is high initially, then becomes insignificant, due to the small surface area of follicles and glands in relation to the total surface area of the stratum corneum. Topical delivery of pesticides to domestic livestock has been used for many years for the control of external as well as internal parasites of these animals, indicating that enough of the pesticide is absorbed systemically to kill some internal parasites while not harming the host animal (Riviere, 1992).

Two events must occur for a drug to be successfully delivered to the epidermis under the stratum corneum: (i) the drug must diffuse out of the vehicle and onto the surface of the stratum corneum; and (ii) the drug must be able to penetrate the stratum corneum (Idson, 1983). Restated, the vehicle in which the topical drug is being delivered should have significantly less affinity for that drug than for the stratum corneum. In actuality, many factors enter into the release of drug from the vehicle and its penetration through the stratum corneum. These factors have been mathematically described for steady-state kinetics in Fick's first law of diffusion (Idson, 1983):

$$dQ/dt = (PC)C_vDA/h$$

where dQ/dt is a steady rate of penetration, PC is the drug's partition coefficient between the vehicle and the stratum corneum, C_v is the concentration of the drug dissolved in the vehicle, D is the diffusion coefficient, A is the area of skin to which the drug is applied, and h is the diffusion path length through the stratum corneum. In short, Fick's law states that the driving force causing the transfer of a drug from regions of high concentration to areas of low concentration is proportional to the concentration gradient (Idsen, 1975). The diffusion coefficient is primarily correlated to the partition coefficient of the penetrant, and can also be predicted from a knowledge of the drug's molecular volume and hydrogen bond activity (Potts and Guy, 1995).

Only the nonionized moiety of a weak acid or base is available for diffusion across the stratum corneum. The pH of skin is variable and sensitive to hydration state. The typical pH of skin ranges from 4.2 to 7.3. For drugs with pK_a values within this range, the ionized fraction may vary according to the principles governed by the Hendersen–Hasselbalch equation, which would alter the amount of drug available for absorption. This is usually controlled for in the dermatological formulation by use of buffering systems. Theoretically, vehicles with different pH may have species-specific absorption for drugs with pKa values in this range.

Topical Vehicles

A vehicle is defined as the medium in which a medicinally active agent is topically administered. It is a gross misconception that the vehicle in which topical drugs are dissolved is inert and has little effect on the skin; in fact, the physical and chemical properties of the vehicle will largely determine how successfully the drug will penetrate the skin. This has been clearly demonstrated with the pesticide carbaryl in pigs (Baynes and Riviere, 1998). The vehicle may penetrate the stratum corneum to some extent and change the solubility of the intercellular lipid matrix. A simplistic view of the fate of topically applied drugs has been outlined in Figure 47.5.

The partition coefficient is a unitless measure of the relative affinity of a compound between a highly hydrophobic phase (usually octanol) and a hydrophilic phase (water), and is a physicochemical factor that has a major role in determining the percutaneous absorption of a chemical. As the partition coefficient increases, the compound has a greater affinity for the lipid phase and less affinity for the water phase. As discussed earlier, the

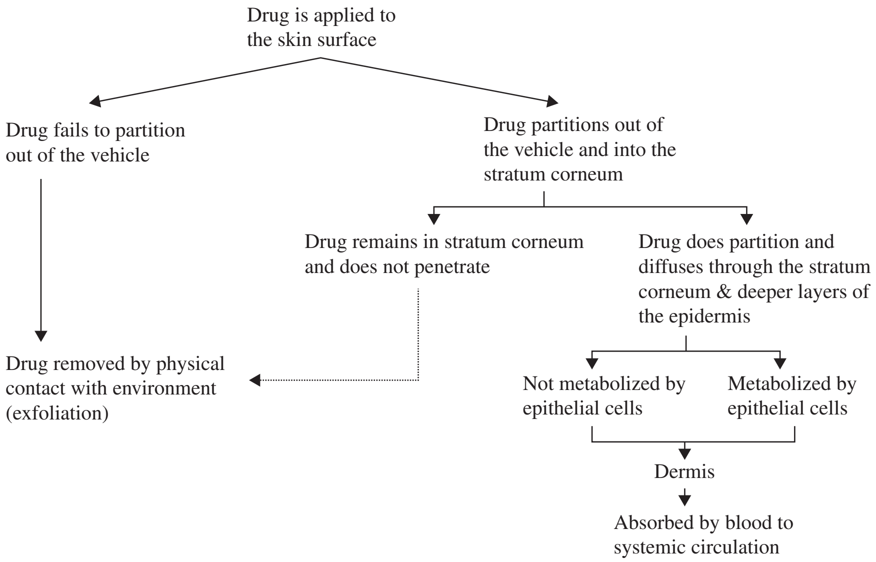
Drug is applied to the skin surface
Drug fails to partition out of the vehicle
Drug partitions out of the vehicle and into the stratum corneum
Drug remains in stratum corneum and does not penetrate
Drug does partition and diffuses through the stratum corneum & deeper layers of the epidermis
Drug removed by physical contact with environment (exfoliation)
Not metabolized by epithelial cells
Metabolized by epithelial cells
Dermis
Absorbed by blood to systemic circulation

Figure 47.5 Schematic representation of the fate of topically applied drugs.

intercellular matrix of the skin consists of several different types of lipid, the ratios of which may vary from site to site, and furthermore be modulated by vehicle penetrating the skin.

Skin penetration of many chemicals has often been correlated to the partition coefficient of the penetrant. Generally, as the log of the partition coefficient increases, lipid solubility increases and there is an overall increase in the percutaneous absorption of the compound through the skin. This scenario is not always the case, however; some poorly lipid soluble compounds (i.e., low partition coefficients) sometimes penetrate the skin much more easily than compounds with higher partition coefficients. Once penetration into the skin occurs, the molecule must eventually leave the lipid phase to be able to reach the systemic circulation. Compounds with high partition coefficients tend to remain within the lipid of the skin and form a reservoir rather than pass through; this is a well-documented phenomenon in skin (Muhammad et al., 2005). It has been shown that a log partition coefficient of 1–4 is desirable for skin penetration of most compounds.

Absorption of the vehicle into the stratum corneum is another factor to consider, since the vehicle may cause some disruptions in the composition and orientation of the intercellular lipid matrix. Partition coefficients for use in predicting the percutaneous absorption of topically applied drugs should ideally measure the index of affinity of a drug between the vehicle the drug is being delivered in and the lipid/vehicle composition of the stratum corneum. Unfortunately, few measurements of this kind are in existence because interaction of the vehicle with the stratum corneum lipids invalidates its determination. The role of partition coefficients in skin has been extensively reviewed elsewhere (Surber et al., 1990a, 1990b; Sloan et al., 1986; Aungst et al., 1990).

The optimal vehicle is one in which the drug is soluble enough to enter into solution, but has less affinity for the vehicle than the stratum corneum lipids, thereby favoring partitioning from the vehicle into the epidermis. Optimal delivery for a polar drug may thus be a relatively hydrophobic vehicle that has sufficient solubility to dissolve the drug. However, if a drug is too soluble in a vehicle relative to the stratum corneum, the drug may persist in the vehicle and only slowly release drug into the skin for as long as the vehicle stays on the skin.

The effect of the vehicle on chemical absorption has been quantitated based on competing physical chemical interactions of the drug and the vehicle versus the skin, using quantitative structure activity relationship principles (Riviere and Brooks, 2005, 2007; Karadzovska et al., 2013a, 2013b; Riviere et al., 2014). These studies also compared topical ectoparasiticides vehicle modulation between canine and porcine skin and has the potential for rationally designing topical drug formulations based on physical chemical principles.

The ability of the vehicle to stay on the skin surface long enough to allow passive absorption of the drug to occur is an important factor in passive drug absorption. The vehicle can be removed by three mechanisms: (i) absorption of the vehicle through the stratum corneum, (ii) evaporation from the skin surface into the surrounding air, or (iii) physical removal (rubbing, licking, scratching, etc.). If the vehicle penetrates the stratum corneum more quickly than the drug, the concentration of the drug in the vehicle left on the surface of the stratum corneum will increase, which would increase the concentration of drug driving absorption, but would also have the potential of inducing precipitation of the drug onto the skin's surface. The vehicle may also change the permeability of the lipid matrix (e.g., function as an enhancer). Evaporation of the vehicle will result in a similar scenario.

Vehicles may also affect the hydration state of the stratum corneum, which in turn may affect the rate at which topically applied compounds can penetrate the stratum corneum. Occlusion of the skin significantly increases the penetration of parathion (Chang and Riviere, 1993) and other compounds. The vehicle can accomplish this by modulating insensible cutaneous water loss or skin transpiration. "Transpiration" is the term used to describe the passage of water vapor from the body, through the epidermis, and out to the surrounding environment. The act of transpiration is an important factor in determining percutaneous absorption of chemicals. In the normal state, the epidermal cells below the stratum corneum are highly hydrated compared to the stratum corneum. The water in and around these epidermal cells tends to slowly "percolate" upward, following the decreasing concentration gradient of water toward the less hydrated stratum corneum, eventually passing through the stratum corneum and into the environment. This phenomenon can be noninvasively assessed by measuring transepidermal water loss (TEWL) and has been correlated to the stratum corneum permeability of hydrophilic drugs. The stratum corneum normally maintains a 10–20% hydration state. When hydration of the stratum corneum occurs, which may reach 60–80% of the total stratum corneum's mass, this water "opens" the compact substance of the stratum corneum and reduces the density of the intracellular structures, thereby decreasing the cells' resistance to passive diffusion and allowing substances to permeate more readily than in the normal, dehydrated state. Hydration of the stratum corneum can be accomplished by slowing the rate of epidermal water loss to the environment by applying occlusive emollients to the skin (petrolatum, lanolin, etc.), by applying a nonwater-permeable membrane (patch), by soaking the skin in water, or by increasing the relative humidity of the air surrounding the skin (Blank et al., 1984). An increase in percent hydration from 10 to 50% in the stratum corneum can result in as much as a tenfold increase in diffusion constants (Idson, 1983). This effect of environmental humidity and occlusion of the absorption of parathion through pig skin was clearly demonstrated (Chang and Riviere, 1993).

Even when fully hydrated, the stratum corneum is one of the most water-impermeable biological membranes found in nature (Idsen, 1975). Similarly, decreasing the hydration of the stratum corneum can decrease percutaneous absorption of substances. Dehydration of stratum corneum cells below the normal 10–15% usually results in "dry skin" and is a secondary symptom of many dermatological diseases. Decreased stratum corneum hydration often results in a rugged and rough feeling to the skin, along with cracking, increased desquamation of the stratum corneum, and possible secondary septic or aseptic dermatitis. Dry skin can be treated by (i) treating the underlying condition causing the disease and/or (ii) applying agents that will increase the hydration of the stratum corneum by slowing transepidermal water loss. Applying keratolytics (discussed in Section Keratolytics, Keratoplastics, and Antiseborrheics) is also of use.

This discussion should point out that selecting a vehicle that increases the amount of water in the stratum corneum will most likely result in more permeation of drug to the underlying affected tissues, an important factor in successful dermatological therapy. In diseases involving dry skin, returning the stratum corneum to its normal hydration state should also be a goal of dermatological therapy.

In summary, the vehicle, not just the choice of drug, plays a crucial role in determining the success or failure of dermatological therapy. No "ideal vehicle" exists; the veterinary clinician must evaluate the patient's skin lesion and should determine what drug is indicated and which vehicle would best augment that therapy. Both factors may markedly influence the success of therapy. Pharmaceutical classifications of topical vehicles are covered in Section Classification Of Dermatological Vehicles.

Other Factors Affecting Percutaneous Absorption

Other factors that may enhance or hinder percutaneous absorption are the molecular weight of the chemical, temperature, blood flow, and skin age. Increasing the blood flow through the dermis would suggest a more rapid removal of drugs absorbed from the epidermis (Riviere and Williams, 1992). Vasoconstriction of these vessels has been shown to significantly decrease percutaneous absorption of 6-methylprednisolone and testosterone (Malkinson, 1958). The temperature of the air surrounding the skin plays a role in blood flow, with warmer environmental temperatures or skin showing one of the cardinal signs of inflammation (heat) increasing the blood flow to the skin and cooler temperatures decreasing cutaneous blood flow. An inverse relationship appears to exist between absorption rate and the molecular weight of the drug (Idsen, 1975). Although smaller molecules tend to increase total topical dose absorbed through the skin, there is considerable variability in the amounts percutaneously absorbed among compounds of similar molecular weights (Idsen, 1975; Tregear, 1966).

It is important to note that skin barrier function is impaired in inflamed skin and in certain conditions such as atopic dermatitis. Thus application of topical products in patients with inflamed or traumatized skin is increased leading to potentially increased risk for adverse effects.

Penetration Enhancers

The stratum corneum has been portrayed as a formidable and almost impenetrable barrier to the absorption of lipophilic, hydrophilic, and amphoteric xenobiotics. Many efforts have been made to adjust the barrier of the stratum corneum to increase the percutaneous absorption of topically applied xenobiotics, for the most part with only limited success. The barrier function of the stratum corneum can be structurally modified using a relatively small class of compounds, collectively known as penetration enhancers or penetration accelerants, that increase the percutaneous absorption of many compounds.

Many reports exist in the human and veterinary literature of various agents that have been shown to accelerate penetration of compounds through the skin, but few describe the precise mechanism by which an individual penetration enhancer performs this function. One theory, the lipid–protein partitioning concept, has been proposed for the mechanism of action of all the known penetration enhancers, including penetration via the intercellular as well as the intracellular routes (Barry, 1991). For both routes, this theory acknowledges that polar (hydrophilic) molecules permeate the skin via polar channels (either by aqueous pores or by alkyl group modulation), which are different from the channels used by nonpolar (hydrophobic) molecules. After application to the skin surface, the drug molecules diffuse out of the vehicle and into the stratum corneum and begin to traverse the many aqueous and lipid barriers found in the intercellular matrix (see Figure 47.4) in order to reach the rest of the body via the systemic circulatory system. Penetration enhancers are hypothesized to modify these lipid and aqueous bilayers of the intercellular lipid matrix and allow topically applied compounds to penetrate the stratum corneum more readily by disrupting the normally very organized structure of the lipid layers.

It is convenient to organize the known penetration enhancers into four common subgroups: azone and its derivatives, urea and its derivatives (1-dodecylurea, 1,3 didodecylurea, 1,3 diphenyl urea, propylene glycol, dimethylisosorbide), the terpenes (carvone, pulegone, piperitone, menthone, cyclohenene oxide, terpinen-4-ol, and others), and the aprotic solvents (dimethylsulfoxide, demethyl formamide, decylmethyl sulfoxide, 2-pyrrolidone, and others). At the present time, the first three groups are used almost exclusively in human pharmaceutical products and research and will not be discussed here. The most extensively used penetration enhancer in veterinary medicine, primarily for transdermal applications, is dimethylsulfoxide (DMSO).

DMSO is a product from the processing of wood pulp and is a dipolar and aprotic solvent. DMSO is also produced by phytoplankton and is present in many foods (Herschler, 1982). DMSO has been used to treat a myriad of skin ailments, including otitis externa, interdigital cysts, lick granulomas, superficial burns, skin grafts, and snakebites; and to reduce the engorgement of the mammary glands of the nursing bitch (Knowles, 1982). Other less common veterinary uses for DMSO have also been described (Jacob et al., 1965; Jacob, 1982; Knowles, 1982). In addition to penetration enhancement, DMSO is also bacteriostatic, vasodilatory, fibrinolytic, antiinflammatory, and produces some degree of topical analgesia. DMSO produces a thermal effect after direct application to the skin, which may function to alleviate pain in the skin and in the underlying muscle and bone. The transient erythema that is induced after topical administration of DMSO is due to the release of histamine (Jacob et al., 1965). These effects are considered transient and reversible and will not generally increase in severity after multiple treatments, indicating no need to discontinue therapy. Potential skin irritation occurs at concentrations of greater than 70% (see previous editions of this text for more information on DMSO).

DMSO has the potential to enhance the percutaneous absorption of a number of topically applied compounds. The exact mechanism of this percutaneous absorption enhancement has not been clearly elucidated. DMSO appears to produce only minor morphological changes in the skin. Penetration enhancement of topically applied chemicals dissolved in DMSO does not appear significant until the DMSO concentrations approach 50–60%, with overall permeability of the compound tending to increase as the concentration of DMSO applied to the skin approaches 100% (Kurihara-Bergstrom et al., 1987). Many mechanisms have been proposed to attempt to explain why DMSO enhances the percutaneous absorption of such a wide variety of compounds. Possible mechanisms include elution of stratum corneum lipids and delamination of the horny layer by stress resulting from crosscurrents of highly water-interactive DMSO and water (Kurihara-Bergstrom et al., 1986). Sharata and Burnette (1988) determined that in nude mice skin, DMSO transformed the highly compact cell contents of the basal stratum corneum (keratin) into a looser meshwork of filamentous bundles, resulting in much more porous intracellular structure with an increase in intracellular surface areas, which they believed might increase percutaneous transport. Whatever the mechanism, DMSO has been shown to enhance the percutaneous absorption of many compounds, including water, hydrocortisone, salicylic acid, tubocurarine HCl, multiple glucocorticosteroids and antibiotics, estradiol, hexachlorophene, phenylbutazone, and a host of other topically applied chemicals.

Finally, recall that there is no universal penetration enhancer or vehicle, for example pluronic lecithin organogel (PLO) gel, that would be optimal for the delivery of all classes of topical drugs across the skin of all species. Carefully controlled studies have begun to show lack of transdermal delivery of several drugs via this approach (Hoffman et al., 2002; Mealey et al., 2004; Miller et al., 2014), though application of methimazole in PLO gel on the permeable inner pinnae of cats was as effective as oral dosing (Hill et al., 2011; Boretti et al., 2013). Many pour-on ectoparasiticides contain, as one formulation component, the glycol ether transcutol (diethylene glycol monoethyl ether) as a solubilizing agent in ethanolic vehicles. Selection of an optimal vehicle for transdermal delivery of a drug requires a close match between the physical chemical properties of the drug, vehicle, and skin lipids.

Electrically Assisted Transdermal Drug Delivery

An alternative approach to overcome the stratum corneum permeability barrier is by using electrical (iontophoresis) or ultrasonic (phonophoresis) energy, rather than the concentration gradient of diffusion, to drive drug through the skin (Riviere and Heit, 1997). These techniques hold the most promise for delivering large drugs such as peptides and oligonucleotides that now can be administered only by intravascular injection. With these techniques, dose is based on the application surface area and the amount of energy required to actively deliver drug across the skin. In iontophoresis, this amounts to a dose being expressed in mAmps/cm^2. Formulation factors are also very different, since many of the components used are also delivered by the applied electrical current in direct molar proportion to the active drug. Finally, a related strategy is to employ very short-duration high-voltage electrical pulses (electroporation) to reversibly break down the stratum corneum barrier, allowing larger peptides, and even small proteins, to be delivered systemically (Riviere et al., 1995). These approaches, if applied to veterinary medicine, could have implications for the use of controlled transdermal delivery as a viable method of drug delivery.

Classification of Dermatological Vehicles

As discussed in the introduction to this chapter, the defining difference between dermatological and other treatment areas of veterinary pharmacology is the topical route of administration coupled with the overriding influence that the dosage vehicle exerts on absorption and activity of the applied drug. Classification of vehicles was introduced in Chapter 5. The vehicles used as veterinary dermatologics have been historically classified into seven broad categories (Harvey, 1985; Block, 1985; Rippie, 1985; Swinyard, 1985; Swinyard and Lowenthal, 1985; Nairn, 1985).

Adsorbents and Protectives

Adsorbents and protectives are used extensively in many veterinary pharmaceutical preparations. Adsorbents act by binding gases, toxins, and some organisms (such as bacteria) to prevent exposure to the damaged skin surface. Protectives function by providing an occlusive layer of protection from the external environment or by providing mechanical support to the affected area. Protectives and adsorbents can be further subdivided into two subclasses: dusting powders and mechanical protectives.

Dusting powders are generally inert and innocuous substances. They include starch, calcium carbonate, talc, titanium dioxide, zinc oxide, and boric acid. Many are used in drug formulations alone or as a vehicle for the delivery of other drugs. If the powder particle has a smooth surface, it will act mainly to prevent friction, thus protecting abraded and raw skin surfaces; powder particles with rough or porous surfaces act on the skin by absorbing moisture. Water-absorbing powders tend to coalesce and eventually occlude the skin's surface when wetted and therefore should not be used on wet, moist, or highly exudative skin surfaces. Powders that also contain starch or other carbohydrates that are allowed to cake onto these moist skin surfaces give bacteria and fungi (mainly *Candida* spp.) a source of energy, allowing them to proliferate. This may lead to a secondary bacterial and/or fungal skin infection or exacerbation of an existing microbial skin infection. Powders should not be used within body cavities (thorax, abdomen) or in skin abscesses, especially those powders containing talc. Talc has been shown to promote severe granulomatous reactions when used in these areas but is relatively innocuous when used on intact skin surfaces.

Mechanical protectives provide an occlusive film that protects the underlying skin from irritants present in the external environment (ultraviolet radiation, contact irritants, toxins), provide mechanical support, and are popular vehicles for many drugs. Many members of this subgroup are used to treat cutaneous ulcers and other hard-to-heal cutaneous wounds. Compounds in this group include kaolin, lanolin, anhydrous lanolin, mineral oil, olive oil, peanut oil, petrolatum, zinc stearate, and recently a number of synthetic polymers.

Demulcents

Demulcents are generally high molecular weight compounds that are water-soluble and function by alleviating irritation (*demulcere,* "to smooth"). Like the compounds listed in the protectives group, demulcents can coat the surface of damaged skin and protect the stratum corneum and underlying cellular structure of the epidermis by forming a protective barrier against the surrounding environment. They differ from protectives in that they inherently reduce the irritation from these external stimuli. This group comprises a large and diverse number of chemicals, including mucilages, gums, dextrins, starches, polymeric polyhydric glycols, glycerin, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, hydroxyethyl cellulose, methyl cellulose, polyvinyl alcohol, and recently silicone-based polymers such as dimethicone. The dried gum extracts from the acacia and tragacanth plants can readily dissolve in water to form mucilages (see previous editions of this text for more detail). The most commonly used demulcents in veterinary dermatology today are glycerin, propylene glycol, and the polyethylene glycols.

Glycerin is a hygroscopic, trihydric alcohol prepared from propylene and has been used for many years as a suppository, with high concentrations of this vehicle relieving constipation by drawing water from the body and into the colon. Glycerin is a clear, colorless liquid that is miscible with water and alcohol and has been used neat to reduce corneal edema and to facilitate ophthalmoscopic examinations. Used in high concentrations topically on the skin, glycerin may dehydrate and irritate the skin, thereby increasing transepidermal water loss. Lower concentrations of glycerin absorbed into the skin hydrate the stratum corneum because of glycerin's hygroscopic nature. Despite its side effects, glycerin makes an excellent vehicle for topical drug delivery when used at lower concentrations.

Propylene glycol (1,2 propanediol) is a hygroscopic, colorless, odorless water-soluble liquid that is miscible with many compounds (water, alcohols, acetone, many volatile oils) and is also bacteriostatic, fungistatic, and nonocclusive. It was first considered for use in 1932 to be used with a drug to treat human syphilis and has also been used as a nontoxic antifreeze in dairies and beer breweries (Catanzaro and Smith, 1991). Propylene glycol is an ideal medium for a formulation component for topical and transdermal delivery of many drugs in animals and humans, as well as for many oral and parenteral drug formulations, such as antitussives and shampoos. Propylene glycol spreads evenly onto the skin's surface and has a very low evaporation rate; it is not greasy to the touch, does not stain clothing or hair, and has some effect on slowing transepidermal water loss, thereby hydrating the stratum corneum to some extent. Topical hypersensitization and other toxicities are frequently seen in clinical practice in patients with predisposition toward allergies. For this reason clinicians need to be aware of potential worsening of disease while using products containing propylene glycol and the need to select alternative formulations. This can be of great importance when selecting topical products for otitis therapy.

Polyethylene glycols are a group of structurally similar compounds that differ in molecular weight. The larger the number, the higher the molecular weight and the more viscous the formulation becomes. At ambient temperature, polyethylene glycols 200, 300, 400, and 600 are clear viscous liquids; polyethylene glycols 900 to 9000 are semihard waxy solids at room temperature. As a group, the polyethylene glycols do not easily hydrolyze and are nontoxic, bland, highly water soluble, and nonvolatile.

Emollients

Emollients are bland, fatty materials often used to soften or moisten the skin. Emollients are particularly useful when treating skin conditions resulting from water-soluble irritants and airborne bacteria because of their ability to act as a protectant, sequestering the damaged skin away from these noxious stimuli. When used topically, emollients soften skin by decreasing transepidermal water loss, or transpiration (discussed in Section Topical Vehicles), and increasing the hydration of the stratum corneum. Therefore, this is a useful group of compounds for treating dermatological conditions involving dry, crusty, or flaky lesions of the epidermis. Emollients are used today as vehicles for many lipid-soluble drugs. Examples of commonly used emollients are listed in Table 47.2.

Astringents

Astringents precipitate protein, toughen the skin, promote healing, and dry the skin when applied topically. When used to coagulate blood, astringents are said to be styptic and elicit a mildly uncomfortable sensation when applied to small open wounds. Most of the chemicals in this group are inorganic salts of aluminum, zinc, potassium, and silver, and include aluminum chloride, aluminum sulfate, calamine (a combination of Fe_2O_3 and ZnO_2), potassium permanganate, silver nitrate, zinc chloride, zinc oxide, zirconium chlorhydrate, and tannic acid. There are many germicidal agents that also have astringent activity. Other astringents are of vegetable origin, most of these preparations owing their activity to tannic acid (gallotannic acid). Astringents in the vegetable-derivative group include gallic acid, kino, krameria, and rubus (blackberry) (see previous editions of this text for more detail). Astringents have limited uses in veterinary medicine today.

Table 47.2 Emollients in use today

Official vegetable oils	Animal fats	Hydrocarbons
Olive oil	Lanolin (wool fat with water added)	Paraffin
Cottonseed oil	Anhydrous wool fat (no water added)	Petrolatum
Corn oil	Lard	White petrolatum (Vasoline)
Almond oil	Whale oil	Mineral oil
Peanut oil		White/yellow waxes (beeswax)
Persic oil		Spermaceti
Cocoa butter		

Rubefacients, Irritants, and Vesicants

Chemicals in this class are used to induce hyperemia (rubefacients), hyperemia and inflammation (irritants), or cutaneous blisters (vesicants). Heat applied to the skin via a hot-water bottle, heat lamp, moist hot pack, or an electric heating pad are acceptable rubefacients and are extensively used in human medicine. Chemical rubefacients are more commonly used in veterinary medicine, mainly due to the difficulty in applying the heat sources listed above for extended lengths of time. An abbreviated list of chemicals that are known rubefacients, irritants, or vesicants includes anthralin, camphor, coal tar, cresote, ichthamnol, menthol, methyl salicylate, resorcinol, cantharidin, black mustard, iodine, mercuric iodide, capsicum, chloroform, alcohols, turpentine, thymol, and pine tar. With the exception of coal tar, most members of the chemical group are not commonly used in modern veterinary practice because of their potential toxicity. Coal tar is a component found in many veterinary dermatology preparations (especially shampoos) and is a by-product of the distillation of bituminous coal. Photosensitization has been reported to occur. Coal tar should not be used on cats, because of frequent irritant or allergic reactions. Camphor is a rubefacient that has been used in some topical preparations and has some mild analgesic activity. It is of limited use in the treatment of mild pruritus. Similarly, menthol stimulates sensory nerve endings, inducing a feeling of coolness, induces mild analgesia, and is also a mild antipruritic (Harvey, 1985).

Caustics and Escharotics

Caustics (also known as *corrosives*) are agents that will destroy tissue after one or more applications. Escharotics are corrosive and will also precipitate proteins, leading to the formation of a scab and eventually a permanent scar. As with the rubefacient, irritant, and vesicant group, many of the caustics and escharotics have few uses in veterinary dermatology today. Historically, caustics have been used to induce desquamation of the stratum corneum (keratolytic) and to treat warts, keratoses, and other hyperplastic skin diseases. Escharotics have also been used to seal cutaneous ulcers and wounds. Examples of this group include glacial acetic acid, alum, aluminum chloride, gentian violet, phenol, potassium hydroxide, salicylic acid, and silver nitrate.

Keratolytics, Keratoplastics, and Antiseborrheics

Keratolytics function by loosening keratin, which facilitates the desquamation of the stratum corneum, whereas keratoplastics attempt to "normalize" keratinization by slowing basal cell proliferation through inhibition of DNA synthesis. Other mechanisms may also contribute to this effect. Antiseborrheics attempt to modulate sebum production in the skin. Many of the keratolytics available today are also keratoplastic and/or antiseborrheic; therefore, these functions are discussed together. Most keratolytics and antiseborrheics are also cutaneous irritants, which may, in some cases, preclude their use on diseased skin in some animals (Shanley, 1990). Several keratolytics and antiseborrheics used in veterinary dermatology are discussed here.

Salicylic acid (2-hydroxybenzoic acid) is used topically as a keratolytic, keratoplastic, and antiseborrheic agent and has some mild antibacterial and antifungal actions. Salicylic acid (2–10%) causes the dead or dying cells of the stratum corneum to hydrate, swell, and soften, thereby hastening their desquamation, and solubilizes the intercellular lipid layer, releasing the cells of the stratum corneum from each other. Salicylic acid is used extensively in veterinary dermatology to treat many keratoproliferative diseases. Topical use of salicylic acid may cause an irritant dermatitis in animals and has been studied extensively in both humans and many animal models (Weirich, 1975; Nook, 1987; de Mare et al., 1988; Sloan et al., 1986; Roberts and Horlock, 1978).

Benzoyl peroxide is a keratolytic and antiseborrheic agent that is also a strong oxidizer, free-radical generator, and antimicrobial and has been used in veterinary and human dermatology for many years. It important to note that products for people contain higher concentrations of benzoyl peroxide (5–10%) than the veterinary products (2.5–3%). Thus human products should *not be used* on animals due to the increased sensitivity of animals to the irritant properties of this ingredient. The majority of topically applied benzoyl peroxide is metabolized to benzoic acid by the viable epidermal cells during penetration through the skin (Holzmann et al., 1979; Nacht et al., 1981). Benzoyl peroxide in high concentrations can cause skin irritation and was identified as being a skin tumor promoter in SENCAR mice if used in tandem with 7,12-dimethylbenz(a)-anthracene (Slaga et al., 1981; Epstein, 1988; Schweizer et al., 1987). The bactericidal activity associated with benzoyl peroxide comes from its ability to generate free radicals, which disrupt bacterial cell membranes. Due to its bactericidal activities, benzoyl peroxide is indicated in the treatment of pyodermas (Ihrke, 1980a, 1980b) as well as in diseases that cause keratosis. Benzoyl peroxide is believed to have follicular flushing properties and it is useful in diseases with comedone formation such as demodicosis and actinic dermatoses. Transient stinging or burning sensations after application to the affected site have been reported in humans.

Resorcinol (*m*-dihydroxybenzene) is a keratolytic agent that also has bactericidal and fungicidal properties. Like urea (discussed below in this section), it acts as a protein precipitant and promotes hydration of the keratin, resulting in its keratolytic function. Resorcinol may be used alone or compounded with other mild keratolytic agents, such as sulfur or salicylic acid.

Sulfur is a keratolytic, keratoplastic, and antiseborrheic agent used in a variety of topical agents. Sulfur is also antibacterial and antipruritic and has a mild follicular flushing action. The keratolytic activity of elemental sulfur is thought to be related to an inflammatory process that ultimately causes a sloughing of the stratum corneum. The formation of hydrogen sulfide and pentathionic acid is responsible for some of its keratolytic and antibacterial properties. The keratoplastic effect is most likely similar to that of the coal tars, being cytostatic (Miller, 1986). Retinoids are a class of naturally occurring or synthetically manufactured compounds that have vitamin A-like (retinoic acid) activity. Retinol (vitamin A alcohol) is the most potent analog and is metabolized to two other retinols, retinal and retinoic acid. In the early 20th century, researchers documented the importance of vitamin A in reproduction, vision, and epidermal cell growth promotion, along with its functions in differentiation and maintenance of epithelial cell surface structure, including that of the skin (Kwochka, 1989). It was also found that *some* dyskeratotic skin diseases could be correlated to low levels of vitamin A, which eventually led to the therapeutic use of vitamin A to treat these skin diseases. The use of vitamin A was limited, however, as the levels needed to induce a clinical improvement of the lesions often induced signs of hypervitaminosis A. This side-effect dilemma prompted research into vitamin A substitutes, which led to the discovery and testing of over 1,500 retinoids. Three retinoids – tretinoin, isotretinoin, and etretinate – are available for clinical use today.

The retinoids, like vitamin A, act as growth and differentiation regulators in the skin when administered orally or topically. Although the exact mechanism by which the retinoids exert these effects is not completely understood, it is theorized that retinoids alter RNA synthesis within the cell, in turn altering protein synthesis and prostaglandin production, and also affecting some enzymes, such as ornithine decarboxylase and collagenase (Power and Ihrke, 1990). The overall effect of the retinoids is to “normalize” the epithelium in those diseases responsive to these drugs.

In humans, the retinoids are used to treat severe recalcitrant cystic acne, psoriasis, Darier’s disease, pityriasis rubra pilaris, and various other disorders of keratinization. Retinoids also have an ability to control malignant transformations in some tissues that are caused by chemical carcinogens, ionizing radiation, growth factors, and viruses. Naturally occurring retinoids have been shown to protect animals against skin papillomas and carcinomas of the skin and other organs (Griffiths and Vorhees, 1994; Kwochka, 1989). Numerous side effects have been associated with retinoid use in humans, including cheilitis, xerosis of the skin, pruritus, dryness of mucous membranes, epistaxis, thinning of the hair, palmoplantar desquamation, conjunctivitis, headache, ataxia, fatigue, psychological changes, and visual disturbances. Tetragenicity is a serious problem in women who take isotretinoin during pregnancy. Symptoms of hypervitaminosis A may also occur, which is characterized by demineralization and thinning of the long bones, cortical hyperostosis, periostitis, and premature closure of the epiphyses (Kwochka, 1989).

Retinoids are not used to a great extent in veterinary medicine (Werner and Power, 1994). Reported uses of any of the retinoids in dogs and cats are few, and the recommendations for use and possible toxicities and side effects are based on a very small number of animals. Indications for veterinary use remain rather vague, in part because the skin diseases that retinoids may be efficacious in treating in humans do not have exact analogs in domestic animals. There is also a lack of formal studies conducted on large populations of dogs or cats. Reported uses for the retinoids in domestic animals are in disorders of keratinization, which may include primary idiopathic seborrhea in Cocker Spaniels, sebaceous adenitis, canine lamellar ichthyosis, Schnauzer comedo syndrome, and epidermal inclusion cysts, among others (Power and Ihrke, 1990; Kwochka, 1989). The use of retinoids in cats with neoplastic disorders, specifically squamous cell carcinoma and epidermal dysplasia, has been investigated and found to be of limited value (Evans et al., 1985). Toxicities to the retinoids in animals seem to be few, but it should be made clear that the reported side effects and toxicities are based on a small population of clinically ill animals treated for short periods of time with these compounds and these observations may prove to be inaccurate if the use of these compounds increases. Out of 29 dogs treated with isotretinoin in one study (Kwochka, 1989), four dogs developed conjunctivitis that was observed to be reversible after treatments ended. There is no formal information available as to the existence of skeletal anomalies in dogs given retinoids for long periods of time (Power and Ihrke, 1990). Birth defects involving the central nervous system, skeleton, thymus, and heart do occur, as seen with high doses of vitamin A during pregnancy. Cats appear to have a higher incidence of side effects associated with retinoid treatment. Cats treated with isotretinoin were observed to develop periocular erythema, periocular crusting, epiphora, and blepharospasm (Kwochka, 1989).

Isotretinoin (Accutane, Roche; 13-*cis*-retinoic acid) and etretinate (Tegison, Roche) are two systemically used retinoids that also have potential veterinary applications. Power and Ihrke (1990) describe several cases where these two retinoids have been used to treat several skin diseases in dogs, with variable success. Isotretinoin is the most effective inhibitor of sebum production known (Kwochka, 1989), making it potentially useful in treating primary idiopathic seborrhea and comedo syndromes. Although retinoids are associated with less toxicity than vitamin A, toxicities can occur. Etretinate may be associated with fewer toxic side effects than isotretinoin. Insufficient data are available for dogs or cats to determine its efficacy or toxicity.

Coal tar is another pharmaceutical that has keratolytic as well as keratoplastic and antiseborrheic activity. Its mechanism of action and general uses have been discussed previously. Coal tar products should not be used in the feline due to frequent irritant reactions and increased risk of toxicity.

Urea is a product of protein metabolism that, when used topically, acts as a protein denaturant, promoting the hydration of keratin. Once the treated areas of keratin swell, mild keratolysis ensues. The use of urea alone as a keratolytic agent is not common in veterinary dermatology today.

Selenium sulfide is an externally applied antiseborrheic, keratolytic, and keratoplastic compound that also has some antidandruff activities. Selenium sulfide exerts these actions by its antimitotic activity, slowing cell proliferation and sebum production, and tends to be irritating (especially if used in long-term treatment protocols) and also stains hair. It also causes irritation of the mucous membranes, so care should be taken to avoid contact with these tissues. Percutaneous absorption is minimal if applied to intact skin. Selenium disulfide is very useful in treating oily seborrheic disease caused by Malassezia. This ingredient is well tolerated by dogs but should not be used on cats.

Classes of Medicated Applications

Pharmaceuticals can also be classified by the type of base in which they are formulated. There are eight classes of medicated applications (Harvey, 1985; Block, 1985; Rippie, 1985; Swinyard, 1985; Swinyard and Lowenthal, 1985; Nairn, 1985).

Ointments

Ointments are semisolid preparations that usually (but not always) contain drugs used to treat dermatological diseases. There are five classes of ointments commonly used in veterinary medicine.

Hydrocarbon Bases

These emollient ointment bases usually are composed of vegetable oils and animal fats. Common members of this subset include spermaceti, cetyl esters wax ("synthetic spermaceti"), oleic acid, olive oil, paraffin, petrolatum, white petrolatum, white wax, and yellow wax. Hydrocarbon base ointments are emollient and generally hydrophobic and occlusive, causing an increase in the hydration of the stratum corneum and underlying epidermal cell structure by decreasing transepidermal water loss, making them useful in rehydrating or softening the skin. Disadvantages include greasiness and their ability to stain clothing; also, they cannot be washed off in water, making them difficult to completely remove from the skin.

Anhydrous Absorption Bases

This type of ointment base contains very little (if any) water after preparation but differs from the hydrocarbon bases in that it will readily accept any water molecules it comes in contact with. Absorbent ointment bases can absorb large quantities of water and still keep their thick consistency. Examples of this subgroup include hydrophilic petrolatum and anhydrous lanolin, both of which are emollient, occlusive, and greasy.

Water–Oil Emulsion Bases

Emulsions, by definition, are oil and water combinations. Water-in-oil emulsion bases ("creams") are water-washable bases that are easily removed from the skin surface and contain more oil than water on a percentage basis. The oil phase usually consists of petrolatum or liquid petrolatum and perhaps an alcohol (cetyl or stearyl alcohol). The aqueous phase may consist of water; however, other aqueous vehicles may be used, such as propylene glycol, polyethylene glycol, or glycerin, to which various preservatives (such as paraben derivatives) are usually added. Water-in-oil bases are emollient (due to the higher percentage of oil than aqueous phase), occlusive, greasy, and may absorb some water, but not to the same degree as the anhydrous absorption bases. Other common components of water-in-oil emulsion bases include glyceryl monosterate and stearic acid.

Oil–Water Emulsion Bases

Oil-in-water emulsions are manufactured in the same manner as the water-in-oil bases, with the exception that the aqueous phase is in a higher percentage than the oil component. The same ingredients are also used to make the oil-in-water bases. Because of the higher water (or other aqueous phase) content, oil-in-water bases are water washable, nongreasy, and nonocclusive.

Water-Soluble Bases

As the name implies, these bases have lost their hydrophobic lipid base components. These demulcent bases are primarily composed of polymers that are completely water soluble and usually anhydrous, do not easily hydrolyze, do not support mold growth, and are nongreasy, nonocclusive, and nonvolatile. If the components of a preparation contain water-soluble bases in a gelled medium, they are referred to as *gels*. Gels are a combination of propylene glycol, propylene gallate, disodium EDTA, and carboxypolymethylene that results in a clear, water-miscible, and relatively greaseless formulation. A commonly used drug in a gel formulation is DMSO (Domoso, Diamond), discussed in Section Penetration Enhancers. Among other drugs, glucocorticosteroids may also be formulated in this way.

Poultices

A poultice (or cataplasm) is a soft moist mass of materials applied locally to an affected area and was historically composed of roots, herbs, seeds, and even mud in a gruel-like base. The poultice was intended to be a topical wound treatment serving as a counterirritant and absorptive/adsorptive sink. Poultices are rarely used in veterinary medicine today.

Pastes

Pastes are absorptive powders placed in a gelatinous base, usually petrolatum or hydrophilic petrolatum. Pastes have been used to adhere to the skin and thereby act as a “sponge” to absorb exudates and moisture and also as a physical barrier to protect the skin from the external environment. Pastes are easily removed from the skin and can be used on moist lesions of the skin.

Powders

Powders have been discussed in Section Adsorbents and Protectives as a class of vehicle for the delivery of topical drugs to the skin. Powders are commonly used in veterinary medicine to deliver pesticides (carbaryl, permethrins, etc.) for the control of external parasites (mainly fleas) and in large animal veterinary dermatology to deliver antibiotics, such as nitrofurazone, to wounds.

Dressings

Dressings are external applications of some previously discussed compounds (petrolatum, ointments) on an application device such as plastic wrap or sterile gauze and are placed over wound sites to protect the skin lesion from external environmental trauma. Dressings may also contain antimicrobial agents, such as nitrofurazone.

Plasters

Plasters are similar to dressings; however, they are attached to the skin via some adhesive material. They protect skin lesions from the external environment and provide an occlusive environment. Plasters have limited use in veterinary medicine today.

Suspensions

A suspension is a two-phase system composed of a finely divided solid that is dispersed in a liquid, usually water. Suspensions are not utilized to any great degree in veterinary dermatology today. They are more commonly used in oral drug preparation schemes. The most common type of suspension currently used topically is captan (Orthocide, Chevron Chemical), which is used to treat some types of superficial fungal infections and which also has some limited bacteriostatic properties.

Lotions

Lotions are powders dissolved in a liquid, usually water or an alcohol. Lotions, like powders, tend to be cooling, drying, and somewhat mildly antipruritic. Lotions have limited applications in veterinary medicine but are used extensively in human over-the-counter skin-care products.

Dermatotherapy: Atopic Dermatitis

One dermatological disease – atopic dermatitis – deserves specific mention because it is a common clinical syndrome with various stages of presentation and potential initiating etiologies. A systematic review of randomized controlled trials (RCTs) for canine atopic dermatitis reviewed a total of 49 RCTs, which had enrolled a total of 2126 dogs (Olivry et al., 2010). This review found some evidence of efficacy for topical tacrolimus (three RCTs), topical triamcinolone (one), oral glucocorticoids (five), oral cyclosporine (six), subcutaneous recombinant gamma-interferon (one), and subcutaneous allergen-specific immunotherapy (three) to decrease pruritus and/or skin lesions of atopic dermatitis in dogs as monotherapy. One high-quality RCT showed that an oral essential fatty acid supplement has a significant steroid-sparing effect, so although essential fatty acids may not be sufficiently strong to be used as monotherapy, they have a positive effect in decreasing the prednisolone consumption by approximately half. Chapter 29 should be consulted for a complete introduction to glucocorticoids.

When interpreting the results of reviews aimed to make recommendations based on the level of evidence for efficacy of a treatment, it is important to note the difference between reporting evidence of efficacy on a review and the clinical usefulness of such treatment. For example, although evidence of efficacy has been found for interferon, this is not a treatment used in the clinics. Similarly, although evidence was not found for drugs such as antihistamines (a class of drugs fully discussed in Chapter 19), these are still commonly used in the clinics (Zur, 2002) and often with good client satisfaction (Dell, 2012). Antihistamines are best used for their steroid-sparing effects rather than as monotherapy. The lack of reported evidence is frequently due to the lack of large controlled clinical trials and the unrealistic expectation of using antihistamines as monotherapy. Thus the lack of evidence of efficacy reported in the metaanalysis should not be equated with a lack of clinical benefit. It is still recommended to try different antihistamines to find the most helpful in individual cases and to use them in conjunction with other therapies (DeBoer, 2001).

A small more recent trial reported on the beneficial effect of various antihistamines (Eichenseer et al., 2013). In published treatment guidelines for canine atopic dermatitis (Olivry et al., 2015), the importance of controlling both the acute flare ups and prevent more flare ups in the long term is highlighted. The best control of symptoms is typically achieved with a *multimodal approach,* which involves topical therapy (with the intent to both remove the allergen from the coat, decrease inflammation and pruritus with topical glucocorticoids, and improve skin barrier function with moisturizers and topical ceramides) and systemic therapy. With this approach the clinician aims to decrease the level of pruritus below the threshold of clinical signs. Using a multimodal approach, it is common to use lower doses of medications than would be needed to control signs if they were used alone. Additionally, it is important to remember that in many patients environmental allergens absorbed epicutaneously function as important triggers. Thus the removal of those allergens on a routine basis in conjunction with mild hypoallergenic products that improve, or minimally do not worsen, the skin barrier is of great help in many patients. Drugs used for the acute flare ups include topical and oral glucocorticoids and oral oclacitinib (Olivry et al., 2015). Within the topical options, oatmeal and topical anesthetic like pramoxine are frequently used both as shampoos and as leave-on conditioners. In cases of more severe pruritus, topical triamcinolone (triamcinolone 0.015%, Genesis®, Virbac) has been used with good clinical response and minimal adverse effects with prolonged use (DeBoer, 2002). This product is currently not available on the market. Another topical glucocorticoid (1% hydrocortisone, Resicort®, Virbac,) has been used frequently in clinical practice. This product also is currently not available on the market. In Europe, the efficacy of a 0.0584% hydrocortisone aceponate spray (Cortavance(®); Virbac) and cyclosporine (Atopica(®); Novartis Animal Health) in canine atopic dermatitis was found to be comparable in a single-blind randomized controlled trial (Nuttall et al., 2012). Similar proportions of dogs treated with hydrocortisone aceponate spray or cyclosporine achieved ≥50% reductions in CADESI-03 and pruritus scores at 28 days, providing a topical option for affected dogs. This product is not available at this time in the USA.

The long-term effect of topical glucocorticoids is cutaneous atrophy; thus the short-term benefit of controlling pruritus and inflammation is exchanged with the potential for cutaneous atropy if the product is used too often. An alternative option to topical glucocorticoids is the use of topical tacrolimus, discussed in Chapter 45. This approach was developed in human medicine to address the issue of cutaneous atrophy seen with the steroids. Tacrolimus is a calcineurin inhibitor that does not induce cutaneous atrophy and actually improves skin barrier function (Chittock et al., 2015; Dähnhardt-Pfeiffer et al., 2013). One of the undesirable adverse effects of tacrolimus is the stinging and burning sensation that is frequently observed at the beginning of treatment. While in most cases this is a transient adverse effect, many patients elect to discontinue therapy. Another undesirable characteristic of topical tacrolimus is the fact that it comes formulated as a thick ointment that it is messy and greasy on application. The efficacy of tacrolimus for canine atopic dermatitis has been evaluated. A significant reduction in lesional scores has been reported in dogs with localized disease (Bensignor and Olivry, 2005) after 6 weeks of therapy using the 0.1% formulation. Dogs with localized disease respond better than dogs with generalized disease (Marsella et al., 2004a). While the efficacy of tacrolimus was found to be good, the fact that in many dogs the disease was localized to the feet and that this treatment would trap dirt on the hair, the compliance and satisfaction of owners was diminished by the logistics and cosmetics of the treatment. Although some absorption occurs after topical administration, levels were well below toxicity thresholds and this formulation was well tolerated in dogs (Marsella et al., 2004a). In contrast to glucocorticoids, which suppress intradermal skin test reactivity, tacrolimus has no significant effect on immediate reactions after intradermal injection of allergens; thus no withdrawal is necessary prior to testing in patients receiving topical tacrolimus (Marsella et al., 2004b).

Several studies have been conducted on the efficacy of sphingolipid emulsions containing a mixture of ceramides and fatty acids. These products have been shown to improve skin barrier of atopic dogs both ultrastructurally, as shown on electron microscopy, and functionally, as measured by transepidermal water loss (Jung et al., 2013). The improvement of skin barrier corresponds to an improvement of clinical signs (Jung et al., 2013). Normalization of the ultrastructure of the epidermis after this form of treatment occurs parallel to normalization of epidermal lipids (Popa et al., 2012). It is important to note that this type of therapy is most successful when considered as adjunctive therapy rather than as sole therapy and when used for at least a month (Fujimura et al., 2011).

As stated in the published guidelines on the treatment of canine atopic dermatitis (Olivry et al., 2015), it is important to both control acute flare up and have a long-term approach to minimize the occurrence of flare up. For the long-term approach, the most effective therapy still remains allergen-specific immunotherapy. This approach has been shown in human medicine to alter the course of the disease and minimize development of new allergies.

Oral glucocorticoids have been traditionally used to control signs of atopic dermatitis. The most commonly used are prednisone and prednisolone, either alone or in combination with trimeprazine (Paradis et al., 1991). A synergistic effect of prednisolone and trimeprazine has been postulated since a decreased dose of prednisolone is sufficient to control signs when used in combination. With long-term use of glucocorticoids, decreased efficacy is common, necessitating a change to a different form of therapy.

A new and very effective drug for the treatment of canine atopic dermatitis is oclacitinib, a JAK1 inhibitor. Janus kinases (JAK) are tyrosine kinases responsible for modulating cytokine production in some cells. Oclacitinib was shown to be very selective for JAK1 and some effect on JAK3 but no effect on JAK2. Thus the effect is targeted on the suppression of cytokines involved in inflammation and allergy (IL-6, IL-2, IL-4, IL-13) but no effect on cytokines involved in hematopoiesis (Gonzales et al., 2014). Oclacitinib does not have any of the adverse effects reported for JAK inhibitors used in human medicine. Oclacitinib is rapidly and well absorbed after oral administration with a peak concentration within the first hour and a bioavailability of 89% (Collard et al., 2014). Food does not interfere with its absorption and no drug interactions have been reported at this time.

In a clinical trial using privately owned dogs diagnosed with allergic dermatitis, oclacitinib had rapid onset of efficacy, being more effective than placebo starting after the first 24 hours of administration based on pruritus evaluation done by owners (Cosgrove et al., 2013b). By the end of the first week (when dogs were rechecked and evaluated by the veterinarian), significant differences were recorded for both pruritus and skin lesions. The same excellent response was confirmed when oclacitinib was administered to dogs specifically diagnosed with atopic dermatitis and evaluated by dermatologists (Cosgrove et al., 2013a). Dogs were randomized to receive either oclacitinib (0.4–0.6 mg/kg twice daily for 14 days and then once daily for up to 112 days) or an excipient-matched placebo. On days 14 and 28, dermatologists recorded a 48.4% reduction in CADESI-02 scores in oclacitinib-treated dogs compared with a 1.7% reduction and a 3.6% increase in placebo-treated dogs. After day 28, >86% of all placebo-treated dogs had moved to an open-label study.

When the efficacy of oclacitinib was compared to existing therapies such as oral prednisolone (0.5–1.0 mg/kg once daily for 6 days, then every other day) it was found that both treatments produced a rapid onset of efficacy within 4 hours. The mean reductions in pruritus and dermatitis scores were not significantly different between the treatments except on day 14, when reductions were more pronounced for oclacitinib than prednisolone, based both on owner pruritus and veterinarian dermatitis scores (Gadeyne et al., 2014). Adverse events were reported with similar frequency in both groups. When oclacitinib was compared to oral cyclosporine, it was found that oclacitinib had a faster onset of action and a lower frequency of gastrointestinal side effects compared with cyclosporine (Little et al., 2015).

Oclacitinib is labeled for use in dogs older than 12 months due to reports of demodicosis when administered to younger dogs. According to the label, this drug is recommended at a dose of 0.4–0.6 mg/kg twice daily for the first 2 weeks, followed by once daily for long-term treatment. A decrease in control of symptoms is very common when switching from the twice to the once-daily administration. In an uncontrolled study on compassionate use of oclacitnib for long-term management of atopic dermatitis (Cosgrove et al., 2015), an improvement in quality of life was reported by owners in over 90% of cases. An increased risk for development of infections is reported for oclacitinib, as it is also seen for long-term use of glucocorticoids and cyclosporine. It is currently not known if oclacitinib increases risk of developing cancer, as the study on long-term use (up to 630 days) was not controlled. With a lack of polyuria, polydipsia is considered an improvement when compared with glucocorticoids, as is the rapid onset of effect and lack of potential for drug interactions relevant with cyclosporine (Gray et al., 2013), and minimal GI adverse effects, which are also an improvement compared to cyclosporine.

Due to issues with availability of oclacitinib, many patients not able to tolerate glucocorticoids are still maintained with oral cyclosporine. This therapy has been available for over a decade (Nuttall et al., 2014; Archer et al., 2014), and demonstrates an overall good profile of efficacy and owner satisfaction (DeBoer, 2014). Chronic cyclosporine administration is generally safe, with vomiting and diarrhea being the most common adverse effects which can be a limiting factor for many patients. In some cases, freezing the capsules may help to decrease vomiting. Administration of metoclopramide may also decrease emesis. Adverse effects in 55% of dogs have been reported in clinical trials. Besides the gastrointestinal problems (seen in 25% of dogs in the first month of therapy), the most common reported side effects are gingival hyperplasia, and papillomatous and psoriasiform dermatitis, which tend to respond to a decrease in cyclosporine dose. Hirsutism can be seen rarely (less than 1 % of reported cases). Monitoring of blood concentrations is not justified since specific blood concentrations are not linked to clinical efficacy. The clinical relevance of the reported effect of cyclosporine on glucose metabolism (Kovalik et al., 2011) is minimal, as is its effect on calcium metabolism (Kovalik et al., 2012). A topical formulation of cyclosporine has been tested with good success and may provide an alternative for dogs that cannot tolerate oral administration (Puigdemont et al., 2013). This was made possible by developing nano-size formulation, which allows for the penetration of cyclosporine in the skin. However, this is not commercially available for prescription in clinical cases.

An antibody against IL-31 has received conditional approval by USDA for the treatment of canine atopic dermatitis as a subcutaneous injection, once a month. Data on its efficacy and safety in dogs has not been published yet, but this treatment modality has great potential since IL-31 is an important mediator of pruritus in dogs (Gonzales et al., 2013).

In summary, the treatment of canine atopic dermatitis requires a multimodal approach tailored to the specific needs of the patient that utilizes both topical and systemic options and relies on the identification and correction of all trigger factors.

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48

Drugs that Affect the Respiratory System

Mark G. Papich

Antitussive Drugs

Cough Reflex

The afferent arc of the cough reflex receives input from sensory nerves in the bronchial and tracheal airways. Airway irritation and inflammation stimulate the afferent nerves, which in turn activate the cough center located in the medulla oblongata.

Mechanism of Action of Antitussive Drugs

The antitussive drugs directly depress the cough center in the medulla. The site of action may be either μ- or κ-opiate receptors (discussed in Chapter 13). Opiate drugs that act on these receptors are effective, but it is not simply because they produce sedation (Morjaria et al., 2012). Either of the opiate receptors may be responsible for the action because both butorphanol (κ-receptor agonist) and codeine or morphine (μ-receptor agonists) can suppress cough (Takahama and Shirasaki, 2007; Gingerich et al., 1983; Christie et al., 1980; Morjaria et al., 2012), but naloxone is capable of antagonizing this effect.

There may be a nonopiate mechanism for some antitussives as well (Morjaria et al., 2012). For example, dextromethorphan is an opiate derivative, but does not have activity at opiate receptors and may bind to sites in the brain that are distinct from opiate receptors. It is continues to be marketed as an over-the-counter (OTC) antitussive for people – apparently based on early clinical trials. Its mechanism is unknown. The neurokinin receptor (NK) may play a role in antitussive activity of such nonopiates. This mechanism has not been well characterized (Takahama and Shirasaki, 2007), but is discussed further with the NK-1 receptor antagonist drugs below. Beta-2 receptor agonists (β_2) will suppress cough in some animals. The effect of these drugs is mediated via bronchodilation. These drugs will be discussed in Section Bronchodilator Drugs (β-Adrenergic Receptor Agonists).

Use as preanesthetics: Opioids are administered as premedications prior to surgery. This provides a sedative effect, analgesia, and also may facilitate intubation. Inhibition of the cough reflex may decrease laryngeal spasms and coughing associated with intubation during anesthesia induction.

Morphine

Morphine and derivatives have opiate-receptor–mediated effects, but also are effective as antitussives. Morphine is a natural derivative of one of the alkaloids of opium (Chapter 13). Morphine is the prototype of an opiate (narcotic) analgesic with good binding affinity for the μ- and κ-opiate receptors. Morphine can be administered at low doses that produce antitussive effects without causing analgesia and sedation. The antitussive dose has been reported to be approximately 0.1 mg/kg, q 6–8 h. Morphine is not regularly used as an antitussive because of the side effects and potential for abuse and addiction in humans. Oral formulations of morphine do not have good systemic absorption in dogs (see Chapter 13).

Codeine (Methylmorphine)

Codeine phosphate and codeine sulfate are found in many preparations including tablets, liquids, and syrups. There are over 50 different combinations and preparations of codeine. However, codeine oral absorption in dogs is low and inconsistent.

Codeine has analgesic effects that are approximately 1/10 that of morphine. Studies in people have shown little benefit from codeine for cough suppression, but the small amount of codeine metabolized to morphine may produce an antitussive effect. In dogs codeine administered orally attains systemic levels of only 4% (KuKanich, 2010), but it is possible that other metabolites may be responsible for the antitussive effect. Despite the occasional use in dogs, the efficacy for treating cough has not been studied. It is available as

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15, 30, and 60-mg tablets and oral syrups (2 mg/ml). In some states (regulated by local regulations), and some countries, codeine in antitussive formulations may be obtained without prescription.

In people, the side effects of codeine at antitussive doses are significantly less than those experienced with morphine. The potential for addiction and abuse is considerably lower. Important side effects include sedation and constipation.

Hydrocodone

Hydrocodone is similar to codeine in mechanism of action, but it is more potent. Hydrocodone is combined with an anticholinergic drug (homatropine) in the preparation Hycodan®, which has been prescribed often for small animals. Pharmacokinetic studies have shown that is absorbed orally in dogs but there is conflicting evidence in dogs on the metabolism to hydromorphone (Benitez et al., 2015). It is possible that the antitussive effect is produced by a different metabolite. Nevertheless, some veterinarians consider it their first drug of choice for symptomatic treatment of cough in dogs. The anticholinergic component (homatropine) is added to discourage abuse rather than for a respiratory indication. Oral doses administered to dogs are approximately 0.22–0.25 mg/kg, q 6–8 h (approximately 1 tablet per 20 kg).

Available Products

Hydodan® is a combination product with hydrocodone bitartrate formulated as 5 mg of hydrocodone + 1.5 mg homatropine. In some countries it is available without an anticholinergic (5 mg). Hydrocodone (5 mg) + acetaminophen (500 mg) is combined in a tablet and used for analgesia (Vicodin). Because of the acetaminophen in these products, they should never be used in cats. In dogs, high acetaminophen exposure may result from the high doses necessary. In the United States recently, hydromorphone was rescheduled by the DEA from Schedule III, to Schedule II, which makes prescribing more restrictive.

Dextromethorphan

Dextromethorphan is not a true opiate because it does not bind μ- or κ-opiate receptors, but studies in people have supported its antitussive effects. More recent investigations have questioned the antitussive properties of OTC formulations.

Dextromethorphan is the ᴅ-isomer of levorphan (the ʟ-isomer, levorphan is an opiate with addictive properties, but the ᴅ-isomer is not). Dextromethorphan produces mild analgesia and modulates pain via its ability to act as an NMDA (*N*-methyl ᴅ-aspartate) antagonist, but this is unrelated to the antitussive action (Pozzi et al., 2006).

Clinical Use

Dextromethorphan has been administered to dogs and cats, but pharmacokinetic studies in dogs indicated that dextromethorphan does not attain effective concentrations after oral administration (KuKanich and Papich, 2004a). After IV injection it produced adverse effects in dogs (vomiting after oral doses, and central nervous system reactions after IV administration). Even after IV administration, concentrations of the parent drug and active metabolite persisted for only a short time after dosing. Therefore, routine use in dogs is not recommended until more data are available to establish safe and effective doses.

Formulations

There are many preparations available without a prescription (OTC) in liquid and tablet form. For example, Vicks Formula 44® and Robitussin® contain dextromethorphan as their active antitussive ingredient. OTC formulations may vary in concentration, but most contain 2 mg/ml, or 15 or 20 mg per tablet. Pet owners should be cautioned that many OTC preparations contain other drugs that may produce significant side effects. For example, some combinations also contain acetaminophen, which can be toxic to cats. Some preparations also contain a decongestant, such as pseudoephedrine, which can cause excitement and other side effects.

Butorphanol (Torbutrol®, Torbugesic®)

Butorphanol is an opioid agonist–antagonist that has been used both as an analgesic and antitussive. It has been a potent antitussive with clinical studies that support its use in dogs (Gingerich et al., 1983; Christie et al., 1980). High doses may induce side effects such as sedation.

Butorphanol is poorly bioavailable because of oral first-pass metabolism. Therefore, in dogs the oral dose is higher (0.55 to 1.1 mg/kg) than the IV or SC dose (0.05 to 0.1 mg/kg). It is administered as frequently as needed to control cough – usually every 6 to 12 hours. In clinical studies the peak effect was rapid after the injectable formulation. After oral administration, the maximum effects were observed for 4 hours but persisted for up to 10 hours in dogs (Gingerich et al., 1983). By contrast, codeine’s effect in dogs is much shorter. It is available as 1, 5, and 10-mg tablets and an injectable solution.

Tramadol (Ultram and Generic)

Tramadol is an inexpensive oral drug used for analgesia. It possesses opiate, serotonin, and α_2 activity. (The pharmacology of tramadol is discussed in more detail in Chapter 13). The active metabolite has opiate activity and is believed to be responsible for most of the analgesia. This drug also has antitussive properties and appears to be well tolerated in dogs, and it is not a controlled substance. However, efficacy for treating cough in dogs has not been evaluated. The recommended oral dose is 5 mg/kg, every 6 hours, but this has not been tested for antitussive efficacy (KuKanich and Papich, 2004b).

Bronchodilator Drugs (β-Adrenergic Receptor Agonists)

The β-adrenergic receptor agonists have a beneficial effect in the treatment of some airway diseases. They are used commonly in people for the treatment of asthma. In animals, they are used for airway disease, allergic bronchitis, and similar diseases such as feline "asthma," recurrent airway obstruction (RAO) ("*heaves*"), and inflammatory airway disease (IAD) in horses. The autonomic properties are discussed in more detail in Chapter 7 of this textbook, and the application to respiratory diseases will be included here.

Mechanism of Action

Bronchial smooth muscle is innervated by β_2-adrenergic receptors. Stimulation of β_2 receptors leads to increased activity of the enzyme adenylate cyclase, increased intracellular cyclic adenosine monophosphate (cyclic-AMP), and relaxation of bronchial smooth muscle (see Chapter 7).

Stabilize Mast Cells

Stimulation of β-receptor activity on mast cells decreases release of inflammatory mediators. The β receptor on mast cells produces a stabilizing effect (inhibition of mediator release) (Chong et al., 2002). There is little effect on other inflammatory cells. Therefore, when airway inflammation is mediated by neutrophils and/or eosinophils, this property of the β agonists may be less important. Cytological examination of fluid from a tracheal wash or bronchoalveolar lavage may help in making this distinction.

Increased Mucociliary Clearance

There is some evidence that β-adrenergic receptor agonists increase mucociliary clearance in the respiratory tract. The clinical significance of this effect has not been established (Norton et al., 2013).

Clinical Use

The β agonists discussed below should be used for short-term relief of bronchospasm. They should be avoided for long-term use as repeated administration may diminish the response because of a change in receptors after prolonged exposure. The receptor changes during chronic administration are a result of down-regulation and desensitization. Concurrent use of corticosteroids may attenuate the desensitization. For management of airway diseases, other drugs (e.g., corticosteroids) can be used for long-term maintenance, and β agonists used short term during acute exacerbations of bronchoconstriction.

Short-Acting Nonspecific Bronchodilators

Epinephrine (Adrenaline)

Epinephrine stimulates α- and β-adrenergic receptors (Table 48.1). It produces pronounced vasopressive and cardiac effects. Epinephrine is considered the drug of choice for the emergency treatment of life-threatening bronchoconstriction, such as during an anaphylactic reaction. The nonspecific stimulation of other receptors and its short duration make it unsuitable for long-term use. The dose used in animals is 10 μg/kg IM or IV administered as a one-time treatment, or repeated in 15 minutes. Formulations are available in either 1 : 10,000 concentration (0.01%, 0.1 mg/ml) or 1 : 1,000 concentration (0.1%, 1 mg/ml). Duration of action is short (less than 1 hour). Norepinephrine (Levarterenol) has similar β_1 effects as epinephrine but fewer α_1 and β_2 effects, and therefore is not suitable for respiratory therapy.

Table 48.1 Relative effects of catecholamines and adrenergic agonists on adrenergic receptors

Drug	α_1	β_1	β_2	DA
Norepinephrine (noradrenaline)	+++	1	0	0
Epinephrine (adrenaline)	+++	+++	++	0
Ephedrine	+++	+++	+++	0
Dopamine (Intropin®)	+++	+++	+	++++
Dobutamine (Dobutrex®)	+	+++	+	0
Isoproterenol (Isuprel®)	0	+++	++++	0
Isoetharine (Bronkosol®)	0	+	+++	0
Metaproterenol (Alupent®)	0	+	++	0
Terbutaline (Bricanyl®)	0	+	+++	0
Albuterol, Salbutamol (Ventolin®)	0	+	+++	0
Clenbuterol (Ventipulmin®)	0	+	+++	0

DA, dopamine.

Isoproterenol (Isuprel)

Isoproterenol is a potent β receptor agonist. It is selective for β receptors with little α-receptor–mediated effects, but the cardiac (β_1) effects make it unsuitable for long-term use. It has been administered by inhalation or injection. It has a short duration of action (less than 1 hour).

Longer-Acting β_2-Specific Drugs

To avoid the adverse cardiac effects of β-adrenergic agonists, drugs have been developed that are more specific for the β_2-receptor (Table 48.1). These drugs are preferred for repeated use or long-term use. These drugs are also incorporated in some of the metered-dose inhalers used to treat bronchoconstriction.

Terbutaline (Brethine®, Bricanyl®)

Terbutaline is similar to isoproterenol for its β_2 activity, but it is longer acting (6 to 8 hours). It may be injected subcutaneously to relieve an acute episode of bronchoconstriction. (The dose in people is approximately 3.5 μg/kg, repeated once per hour.) The oral dose is 2.5 mg/dog q 8 h for dogs, and 0.625 mg (one-quarter of a 2.5-mg tablet) q 12 h for cats (e.g., cats with feline "asthma") (Hawkins and Papich, 2014). It is available as 2.5 and 5-mg tablets and 0.8 mg/ml (820 μ/ml) injection. The injection can be helpful for treatment of acute bronchoconstriction. The dose for injection in cats is 0.01 mg/kg IV or IM. Terbutaline has been used in horses to treat recurrent airway obstruction (RAO), which is a bronchoconstrictive disease in horses analogous to asthma in people. It is not absorbed after oral administration in horses; therefore, injectable preparations must be used.

Metaproterenol (Alupent®, Metaprel®)

Metaproterenol is similar to terbutaline, but the duration of action is shorter (4 hours). It is available as 10 and 20-mg tablets and 2 mg/ml oral syrup. The dose in small animals is 0.325 to 0.65 mg/kg.

Albuterol (Salbutamol) (Proventil®, Ventolin®)

Albuterol is similar to terbutaline and metaproterenol in its action. Doses used in small animals are 20–50 μg/kg up to four times per day. (Dose in people is 100–200 μg/kg four times daily.) It is available as 2, 4, and 5-mg tablets and 2 mg/5 ml syrup. Levalbuterol is the R-isomer of albuterol, and is used in some products. The proposed benefit of administration of levalbuterol compared to the R-, S-racemic mixture of albuterol is that the S-form may have bronchoconstrictive and proinflammatory properties, and a pure R-isomer avoids these problems (Reinero et al., 2009). In cats, the regular use of the racemic form (R and S) of albuterol increased airway inflammation. Therefore, these authors and others have questioned the regular use of racemic albuterol in cats for treating feline asthma (Reinero, 2011; Trzil and Reinero, 2014). On the other hand, in a study in horses, administration of levalbuterol to horses with RAO did not result in greater bronchodilation compared to albuterol (Arroyo et al., 2016). Levalbuterol was an effective bronchodilator in these horses, but the short duration of action is impractical for long-term treatment of RAO.

Clenbuterol (Ventipulmin®)

Clenbuterol was approved by the FDA in 1998 for use in horses. Its use in food animals is illegal, which is discussed in the section on prohibited drugs in food producing animals. Its use has not been reported in small animals. Clenbuterol has been used primarily for the treatment of recurrent airway obstruction (RAO) (Box 48.1) in horses and some studies have demonstrated efficacy (Shapland et al., 1981). However, its bronchodilating ability has been questioned. Compared to other β agonists, such as terbutaline, clenbuterol has lower clinical efficacy because it is only a partial agonist and has lower intrinsic activity (Törneke et al., 1998; Derksen et al., 1987).

Box 48.1 Airway diseases in horses: recurrent airway obstruction (RAO) and inflammatory airway disease (IAD). Source: Data from Ivester and Couëtil, 2014; Couëtil et al., 2016; Mazan, 2015; Pirie, 2014

Formerly called chronic obstructive airway disease (COPD).
Characterized by:

- chronic airway inflammation
- labored breathing
- bronchospasm
- mucus plugs
- tissue remodeling in airways
- neutrophilic inflammation in airways
- bronchial hyperresponsiveness.

Treatments:

- environmental control
- bronchodilators (β_2 agonists)
- corticosteroids
- inhalant medications
- cromoglycate
- phosphodiesterase inhibitors.

Clenbuterol is available as a syrup (Ventipulmin® syrup, 100 ml and 33 ml bottles, 72.5 μg/ml) for horses for oral administration. The dose is 0.8 μg/kg twice daily, but can be increased to 2×, 3×, and then 4× (up to 3.2 μg/kg) if the initial dose is not effective. The duration of effect is 6–8 hours. Prolonged administration is discouraged (Mazan, 2015; Ivester and Couëtil, 2014). Prolonged administration can diminish the effect through receptor tolerance or tachyphylaxis (desensitization of receptors) (Read et al., 2012). Adverse effects in horses include sweating, muscle tremor, restlessness, and tachycardia.

Effect on exercise in horses: There is conflicting evidence on the effect of clenbuterol on exercise and muscle physiology in horses. One study indicated no improvement in aerobic metabolism of horses (Ferraz et al., 2007). On the other hand, it may have a negative ergonomic effect due to decrease in aerobic capacity, time to fatigue, cardiac function, and oxygen consumption. It has an anabolic effect on skeletal muscle but the anabolic effects do not produce greater athletic performance. One study showed that less than 2 weeks of treatment at approved doses did not reveal any adverse effects on equine cardiac or skeletal muscle (Thompson et al., 2012), but a study in rats (Huang et al., 2000) showed that down-regulation of β_2 receptors occurs after 10 days of treatment and results in a decrease in receptor expression in skeletal muscle (35% decrease) and lung (45%). It appears that the effects on equine performance may be controversial, but there is agreement among equine specialists that clenbuterol should not be used solely to enhance athletic performance (Carlos and Davis, 2007).

Prohibition to use in food-producing animals: Clenbuterol is banned from use in food-producing animals, but has been used in livestock illegally as a repartitioning agent. These agents repartition nutrients away from adipose tissue in favor of muscle (Peters, 1989). The repartitioning and anabolic effects are caused by leptin- and adiponectin-mediated effects that produce an increase in muscle mass (Kearns et al., 2006). In food animals, the result is increased carcass weight and an increased ratio of muscle to fat, while reducing the feed required per kg of weight gain. A review of the use of β-adrenergic agonists as growth-promoting agents is discussed in the article by Mersmann (1998).

Clenbuterol residues in food products pose a threat to people (e.g., pregnant women and people with heart conditions). Deaths in humans have been reported as a result of eating beef liver contaminated with clenbuterol residue, making the illegal use of clenbuterol in food animals a regulatory concern to the FDA and other international agencies. The US FDA has established a “zero tolerance” for residues (Chapter 52). In the USA, clenbuterol residues have been highest in competition "show animals". Another unapproved use of clenbuterol is the abuse by people. It is used (abused) for “muscle building” in human athletes and for weight loss. Also called “*Clen*”, it can be found widely on the internet, promoted for use in people for weight loss and muscle development.

Other β agonists used for weight gain: Zilpaterol (Zilmax®) is another β agonist that has been used in cattle. This is an FDA-approved feed supplement for cattle that has been used by cattle farmers globally for nearly two decades to improve cattle’s natural ability to convert feed into lean beef. Zilpaterol is fed at 6.8 grams per ton (90% dry matter basis) for the last 20 days on the sole ration of feed to provide 60 to 90 mg zilpaterol hydrochloride per head per day. There is a 3-day slaughter withdrawal time. In August 2013, the manufacturer withdrew the medication because of concerns about adverse effects in cattle that included difficulty walking, abnormal gait, and hip pain. Animal welfare concerns were publicized and the company withdrew the medication with plans to reintroduce at a later time.

Salmeterol and Formoterol

These drugs are long-acting β agonists that have been used in human medicine, but their use in veterinary medicine has not been reported.

Inhalant Formulations: Nebulized β Agonists

Inhaled aerosols of β_2 agonists are important drugs for treatment of acute bronchospasm in human patients with asthma or allergic bronchitis. Nebulization is the process of creating small droplets of a drug that can be inhaled into the airways. Nebulized agents are delivered via jet nebulizers, ultrasonic nebulizers, and metered-dose inhalers. (Inhalation products are also discussed with the corticosteroids in Section Inhaled Corticosteroids.) Several metered-dose inhalers are marketed for this use in people, such as albuterol (Ventolin®), bitolterol (Tornalate®), terbutaline (Brethaire®), and pirbuterol (Maxair®).

The use of these aerosols has become more common in animals because adapters are available to allow for use by animal owners. In horses, masks are available that allow a metered-dose inhaler to be used (Derksen et al., 1996; Tesarowski et al., 1994). These devices are available for dogs, cats, and horses. For example, the AeroKat® chamber has also been used to deliver these medications to cats.

Albuterol (Torpex®)

In 2002, albuterol sulfate was approved by the US-FDA for horses, indicated for relief of bronchospasm and bronchoconstriction associated with recurrent airway obstruction (RAO). It is an aerosol formulation in a pressurized canister delivered via a specialized nasal delivery bulb. The nasal bulb is inserted into the horse’s nostril and when the horse inhales, the device is activated.

Masks that fit over the horse's nose (discussed in Section Inhalant Formulations: Nebulized β Agonists) also have been used. In horses, doses of 360 or 720 μg delivered by aerosolization caused significant bronchodilation (Derksen et al., 1999). The same author also administered pirbuterol at 600 μg/horse with good results.

For small animals, the metered dose inhaler to deliver albuterol in people has been adapted for use. However, the use of inhaled albuterol in cats with hyperresponsive airway disease is discouraged because it may exacerbate airway inflammation (Reinero, 2011).

Adverse Effects from β Agonists

The most common adverse effects from β agonists involve the cardiovascular system (tachycardia) and skeletal muscles (muscle tremors) (Fox and Papich, 1989). Cardiac effects (rapid heart rate, palpitations, tremors) can be prominent with β_1 agonists and can be produced by β_2 agonists at high doses. When high doses of β agonists have been nebulized to horses, muscle twitching, sweating, and excitement have occurred. β_2-receptor agonists also inhibit uterine motility and should not be used late in pregnancy. However, these drugs have been used therapeutically for this purpose in situations in which it may be desirable to inhibit uterine contractions to delay labor. High doses of β_2 agonists also can produce hypokalemia.

As noted above in the clenbuterol section, these drugs also have properties that affect skeletal muscle. These drugs have been abused in humans and animals for their muscle-building properties. High doses can cause muscle twitching and hyperthermia.

Tolerance with Chronic Use

Regular administration of β agonists can produce drug tolerance, which is a loss in the sensitivity of β-adrenergic receptors owing to down-regulation of receptors. This effect occurs when β-adrenergic drugs are administered regularly for several weeks. Therefore, it is best to rely on these drugs intermittently and allow drug-free breaks in treatment. In animals, the best use of these drugs is for short-term treatment for acute exacerbations of bronchoconstriction and corticosteroids can be used to decrease inflammation for long-term management.

Cromolyn (Sodium Cromoglycate) (Intal®)

Cromoglycate is used occasionally to stabilize mast cells in animals with hypersensitive airways. It is usually administered as a 2% solution for nebulization in the treatment of bronchoconstrictive diseases. It has been used in people to treat asthma and occasionally nebulized to horses via a special mask to treat recurrent airway obstruction (RAO).

Mechanism of Action

Cromolyn inhibits mast cell release of histamine, leukotrienes, and other substances from sensitized mast cells that cause hypersensitivity reactions, probably by interfering with calcium transport across the mast cell membrane. It has no intrinsic bronchodilator action.

Clinical Use

Cromolyn has been used as an aerosol for treating recurrent airway obstruction (RAO) in horses (Thomson and McPherson, 1981) and has improved some clinical measurements in horses with respiratory disease (Hare et al., 1994). It has been administered via inhalation once daily for 1–4 days, which will provide a therapeutic effect for several days. In one report, nebulization of 80 mg once daily for 1–4 days prevented signs of heaves in horses for up to 3 weeks. The recommended dose is 80 mg of a 0.02% solution from an ultrasonic nebulizer, or 200 mg from a jet nebulizer (Couëtil et al., 2016). However, other studies have produced conflicting results. The primary drawback is the route of administration and timing. To be effective, it *must* be administered before the horse is exposed to the allergen (prophylactically). This agent has its effects primarily on mast cells (Hare et al., 1994). Because airway diseases in animals may have neutrophilic or eosinophilic inflammation, this treatment may have limited impact unless mast cells contribute substantially to the disease.

Nedocromil Sodium

Nedocromil sodium (Tilade) is a chemically unrelated drug, but has an antiinflammatory effect via a similar mechanism to chromolyn. It is approved for use in treating asthma in people but use in veterinary patients has not been reported.

Methylxanthines (Xanthines)

The methylxanthines include theobromine, caffeine, and theophylline. Pentoxifylline also is a methylxanthine, but is used for conditions other than asthma. The methylathines – particularly theophylline – have been used as bronchodilators, but the use is no longer common. Once a mainstay in the therapy of human asthma, theophylline use has diminished because of a high incidence of side effects in people and because there are better-tolerated and more effective drugs available. However, occasionally in small animals theophylline is used because it is tolerated well and oral dosing is convenient. There is occasional use of theophylline in horses (and pentoxifylline for other diseases), but the use of theophylline in horses is hampered by adverse effects and racing restrictions. There are no clinical studies that have documented efficacy in small animals; the use is purely anecdotal and doses based on pharmacokinetic studies.

Pharmacological Effects

The methylxanthines are classified as CNS stimulants, but they have a variety of pharmacological effects on various organ systems (Weinberger and Hendeles, 1996). The methylxanthines relax bronchial smooth muscle and produce antiinflammatory effects that are unrelated to the bronchodilating properties. In addition, there are nonrespiratory effects of methylxanthines, which include CNS stimulation, urinary diuresis (mild), as well as cardiac stimulation (mild).

Cellular Basis of Action

The cellular basis of action of the methylxanthines is still not completely understood. The effect reported in the literature is the action to inhibit phosphodiesterase types 3 and 4 (PDE-3, PDE-4). Phosphodiesterase is the enzyme that catalyzes cyclic-AMP to inactive products. Inhibition of the phosphodiesterase enzyme increases intracellular concentrations of the cyclic nucleotide cAMP. Cyclic-AMP inhibits the release of inflammatory mediators from mast cells, has antiinflammatory effects, and produces bronchial smooth muscle relaxation (Barnes, 2003).

Another mechanism of action that may explain the benefits for respiratory disease is via antagonism of *adenosine* receptors. Adenosine causes bronchoconstriction in asthmatic patients, but blocking this receptor also may contribute to the adverse effects.

Antiinflammatory Effects

The antiinflammatory effects of theophylline may occur separately – and at lower concentrations – than the bronchodilating effects (Barnes, 2003). This may occur by decreasing the expression of inflammatory genes. Treatment with theophylline has induced a decreased response to histamine and other inflammatory mediators in the airways. Theophylline's antiinflammatory effects include diminished activity of inflammatory and immune cells – especially eosinophils – in the airways. Eosinophils can play an important role in the bronchial response to inhaled allergens, and they release inflammatory mediators that induce bronchoconstriction and inflammatory changes in the airways.

Table 48.2 Formulations of theophylline

	Brand name	Percent theophylline
Theophylline base	Many	100
Theophylline elixir	Many	100
Theophylline monohydrate		90
Theophylline ethylenediamine (aminophylline)	Many	80
Choline theophyllinate (oxtriphylline)	Choledyl®	65
Theophylline sodium glycinate	Asbron®	50
Theophylline calcium salicylate	Quadrinal®	48

Formulations

Theophylline has been available in several formulations, including, injectable, aqueous solutions, elixirs, tablets, and capsules. The most convenient for small animal use are extended-release forms that allow for twice daily treatment in dogs and once-daily or once every 48 hour treatment in cats. These are discussed in more detail in Section Clinical Use in Dogs and Cats. However, the availability of these formulations has greatly declined. Because theophylline is uncommonly used in human medicine, and there are no approved veterinary formulations, products for use in animals can be difficult to locate. Examples of other forms of theophylline are presented in Table 48.2. These salts are designed to improve solubility and decrease stomach irritation. The theophylline content of these salts must be considered when calculating a dosage regimen.

Pharmacokinetics

After oral administration, theophylline is rapidly and completely absorbed. Systemic availability is 91%, 96–100%, and 100% in dogs, cats, and horses, respectively. Theophylline is metabolized primarily by the liver in all species; only 10% of the dose is eliminated unchanged in the urine. The pharmacokinetics have been studied in several veterinary species and are listed in Table 48.3 with comparisons to people.

Plasma Concentrations and Clinical Monitoring

Theophylline drug plasma assays are available in many laboratories. When theophylline is used long term, monitoring is recommended to establish the optimum dose. In dogs and cats, the accepted therapeutic plasma concentration is 10 to 20 μg/ml, but these values were extrapolated from human studies. In animals, we aim for plasma concentration greater than 10 μg/ml (usually 10–20 μg/ml) for clinical effects. Plasma concentrations greater than 15 μg/ml have been reported to cause clinical adverse effects in horses.

Table 48.3 Theophylline pharmacokinetics and doses

Species	Half-life (hours)	Volume of distribution (l/kg)	Percent absorbed orally	Suggested dose
People	6–8	0.5	90	4 mg/kg q6h
Dog	5.7	0.82	91	9 mg/kg q6–8h, or 10 mg/kg q12h of the extended release formulations
Cat	7.8,11.7 (IV) 14–18 (oral)	0.46, 0.86	96–100	4 mg/kg q8–12h or extended-release at 15 mg/kg (tab) and 19 mg/kg (cap) q24h (q48h may be possible in some cats)
Cattle	6.4	0.815	93	20 mg/kg q12h
Horse	12–15	0.85–1.02	100	5 mg/kg q12h

Antiinflammatory action may occur in patients at concentrations that are below the concentrations usually considered for bronchodilation. For example in people, a concentration of 6.6 μg/ml theophylline caused a reduction in activated eosinophils (Sullivan et al., 1994). In the review by Barnes (2003) the antiinflammatory effects are reported to occur in people at concentrations of 5–10 μg/ml, which is approximately half of the concentration needed to produce bronchodilation.

Side Effects and Adverse Effects

Mild adverse effects include gastrointestinal signs, which includes nausea and vomiting. More important adverse effects of theophylline are attributed to cardiac stimulation. In this regard, theophylline is more potent than either caffeine or theobromine. The cardiac effects may be summarized as increased heart rate, increase in myocardial contractility (mild), improved right and left systolic function, vasodilation (mild), and diuretic (weak). At high doses, cardiac arrhythmias are possible.

CNS stimulation is more likely from caffeine than theophylline or theobromine. However, at high doses, theophylline also can affect the CNS. These are reported less frequently from theophylline use in small animals than in humans, but they can be common in horses. The CNS effects can be characterized by increased alertness, agitation, nervousness, increased activity, and convulsions at high doses. At high doses there is decreased cerebral blood flow (26%), which may be related to the incidence of seizures.

Caution is advised after rapidly intravenous dosing as adverse effects have been described when theophylline is rapidly administered intravenously in horses and dogs. If the drug is administered orally, high peak plasma concentrations are less likely (Munsiff et al., 1988).

Drug Interactions

Theophylline relies on hepatic metabolism by cytochrome P450 enzymes for clearance. Therefore, interactions are possible when other drugs are administered that affect these enzyme systems. The metabolism of theophylline may be inhibited by erythromycin, fluoroquinolone antibiotics (for example, enrofloxacin), and cimetidine. The metabolism may be induced by rifampin and phenobarbital, which may necessitate increasing the dose if these drugs are used together. Activated charcoal will increase the clearance of theophylline and other methylxanthines and is useful for treatment of a toxic overdose.

Clinical Use in Dogs and Cats

Theophylline has been used for the treatment of both cardiac and respiratory diseases in dogs. Some veterinarians have included theophylline in regimens for the management of congestive heart failure, although this has become an outdated treatment because of availability of newer cardiac drugs. (Note that pimobendan and milrinone, discussed in Chapter 21, are also phosphodiesterase inhibitors.) Theophylline is also employed for the management of intrathoracic collapsing trachea and various forms of bronchitis.

Older sustained-release tablets have been studied in dogs and cats (Koritz et al., 1986; Dye et al., 1989). However, these formulations were recently discontinued and the dose recommendations no longer apply. Decreased availability of older sustained-release formulations led to the use of sustained-release tablets and capsules. These have now become unavailable to many pharmacies, which has led to a decline in use. If available, oral administration of sustained-release formulations in dogs at a dose of 10 mg/kg, q 12 h will produce concentrations in dogs within a range considered to be therapeutic (Bach et al., 2004). Studies in cats (Guenther-Yenke et al., 2007), showed that a dose of 100 mg per cat of the tablet, or 125 mg/cat of the capsule (approximately 15 or 19 mg/kg, respectively) every 24–48 hours maintained plasma concentrations in a range considered to be therapeutic.

Clinical Use in Horses

Administration of theophylline to horses has a narrow therapeutic index and it is considered less effective than other agents (Box 48.1). Adverse effects have prevented more common use and, because of the CNS stimulant effect, it is banned from performance horses.

Despite the risk of adverse effects and questionable efficacy, theophylline has been considered for management of RAO in horses. Studies in the 1980s (Button et al., 1985; Errecalde et al., 1984; Ayres et al., 1985; Ingvast-Larsson et al., 1989; Kowalczyk et al., 1984) summarize the pharmacokinetics and effects of theophylline in horses. Oral doses are preferred to avoid the high concentrations from injections. A loading dose of 12 mg/kg has been followed by maintenance doses of 5 mg/kg, every 12 hours. One study demonstrated that affected horses showed decreased wheezing, decreased respiratory effort, and an improvement in $\mathrm{P}_{\mathrm{CO}_2}$ and blood pH following theophylline administration. However, none of the horses returned to normal. There also is evidence that phosphodiesterase inhibitors are not effective in horses with recurrent airway obstruction (Lavoie et al., 2006). Theophylline produces a positive cardiac chronotropic effect and a weak diuretic effect in horses. Toxicosis can occur from large doses and doses administrated rapidly intravenously. Toxic signs include tremors, excitement, tachycardia, and sweating.

Clinical Use in Cattle

Although there is little clinical experience with the use of theophylline in cattle, experimental evidence suggests that it is a poor bronchodilator in this species (McKenna et al., 1989).

Anticholinergic Drugs

Anticholinergic (parasympatholytic/antimuscarinic) drugs such as atropine and glycopyrrolate are effective bronchodilators and N-butylscopolammonium bromide (*Buscopan* injectable solution) has emerged as an effective agent for horses. (The pharmacology of anticholinergic agents was provided in more detail in Chapter 8.) Cholinergic stimulation causes bronchoconstriction. Anticholinergic drugs inhibit vagal-mediated cholinergic smooth muscle tone in the respiratory tract because asthmatic individuals have excessive stimulation of cholinergic receptors. In some people, COPD can be more effectively treated with anticholinergic drugs than by β agonists (Barnes, 2000). Although atropine will provide relief from these effects in many patients, the side effects are unacceptable for long-term use. Adverse effects involve the CNS and gastrointestinal systems.

Use of Anticholinergic Drugs in Horses

Pearson and Riebold (1989) compared the effects of atropine, isoproterenol, and theophylline in horses with RAO (Box 48.1). Atropine at doses of 0.02 mg/kg, IV was better at relieving some signs of RAO (reduction in intrathoracic pressure for example) than either theophylline or isoproterenol. Isoproterenol was more effective in this study than theophylline.

Drug Choices

If anticholinergic drugs are administered, the quaternary amines such as glycopyrrolate (Robinul-V®), propantheline, (ProBanthine®), and isopropamide should be used instead of atropine because quaternary amines are less likely to cross the blood–brain barrier and CNS side effects will be minimized. (This property is discussed in Chapter 46.)

Atropine is acceptable for short-term use and a 5 mg IV dose has been used as a response test in horses suspected of presenting with RAO. Ipratropium bromide (Atrovent®) is a quaternary amine that is an effective bronchodilator in human asthmatic patients. It is administered as an aerosol and is the first anticholinergic drug to be approved as a bronchodilator. Since ipratropium is not absorbed from the airways, it is described as a "topical form of atropine." It has been administered to horses for treatment of RAO and produced effects for 6 hours at a dose of 2 μg/kg.

N-butylscopolammonium bromide (Buscopan® injectable solution)**:** This anticholinergic, antimuscarinic agent is a quaternary ammonium compound approved by the FDA for treatment of some forms of colic in horses (discussed in Chapter 46). Adverse effects include transient tachycardia and decreased intestinal motility. Because of its antimuscarinic action, it has been administered to horses with RAO at a dose of 0.3 mg/kg IV as a single dose. It has a rapid onset (within 2 minutes) and has a short duration of action that inhibits intestinal motility for only 2 hours (Couëtil et al., 2012).

Adverse Effects

The cardiac effects and inhibition of gastrointestinal function prohibit long-term use of atropine. The topical drugs (e.g., ipratropium) have less systemic effects. Because these agents are used in horses, the most important concern is the effect on intestinal motility. Atropine should not be used repeatedly in horses because it can cause ileus. N-butylscopolammonium bromide has become preferred for the acute treatment of bronchoconstriction in horses because when administered once (0.3 mg/kg IV), it was shorter acting, and produced fewer adverse effects, including those on the heart and intestine, than atropine (de Lagarde et al., 2014).

Glucocorticoids

Glucocorticoids decrease inflammation associated with inflammatory pulmonary diseases. Prednisolone has been effective for treatment of feline tracheobronchitis ("feline asthma"), nonseptic pulmonary diseases associated with pulmonary infiltrates of leukocytes (noninfectious bronchitis), allergic bronchitis, and recurrent airway obstruction (RAO) and inflammatory airway disease (IAD) of horses. For treatment of asthma in people, inhaled corticosteroids are recognized as the most effective therapy available (Barnes, 1995b, 2006). In one article, they were called the "drugs to beat" (Barnes, 2006), and have become the mainstay of asthma management for people.

Mechanism of Action

The pharmacology of the glucocorticoids was discussed in more detail in Chapter 29, with some of the immunosuppressive properties discussed in Chapter 45. In patients with inflammatory airway diseases, glucocorticoids have potent antiinflammatory effects on the bronchial mucosa. Glucocorticoids bind to receptors on cells and inhibit the transcription of genes for the production of mediators (cytokines, chemokines, adhesion molecules) involved in airway inflammation (Rhen and Cidlowski, 2005). In horses with RAO, administration of oral dexamethasone in combination with feeding changes decreased the gene expression of proinflammatory cytokines in bronchoalveolar cells (DeLuca et al., 2008). A decrease in the synthesis of inflammatory mediators such as prostaglandins, leukotrienes, and platelet-activating factor caused by glucocorticoids also may be important (Barnes, 1995a, 1989, 2006). Glucocorticoids have a more pronounced effect on neutrophils and eosinophils than mast cells, which is important because neutrophilic and eosinophilic inflammation has been cited as an important component of equine and feline airway disease (Reinero, 2011; Trzil and Reinero, 2014; Mazan, 2015; Couëtil et al., 2016; Pirie, 2014). Glucocorticoids also enhance the action of adrenergic agonists on β_2 receptors in the bronchial smooth muscle, either by modifying the receptor or augmenting muscle relaxation after a receptor has been bound. Corticosteroids may prevent down-regulation of β_2 receptors. Glucocorticoids and theophylline used together appear to be synergistic (Barnes, 2003).

Clinical Use

Dogs

Oral prednisolone or prednisone is usually the drug of choice when a corticosteroid is needed for treating chronic airway disease. A typical antiinflammatory dosage is 0.5–1.0 mg/kg. After an initial course of treatment many patients can be managed by administering this dose on an every-other-day (EOD) basis. Inhaled corticosteroids also can be administered, such as fluticasone delivered with a metered-dose inhaler (for example, 110 μg twice daily). The inhaled agents are discussed in Section Inhaled Corticosteroids.

Cats

Because cats are somewhat resistant to glucocorticoids, higher doses have been used compared to doses administered to dogs. Oral prednisolone initial dosages of 2–4 mg/kg/day have been used for 10–14 days, followed by chronic doses of 1.0 mg/kg/day for the treatment of feline asthma (Box 48.2). For cats, prednisolone should be used instead of prednisone because of an absorption/conversion problem similar to horses (see Section Horses) (Graham-Mize and Rosser, 2004). For difficult to medicate cats, some veterinarians have administered 20 mg per cat of a long-acting formulation, methylprednisolone acetate (Depo Medrol®) intramuscularly. The effects of one injection may persist for 3 weeks.

Box 48.2 Treatment options for feline asthma

Corticosteroids:

- Prednisolone 1–2 mg/kg q 12 h initially, then taper to lower dose
- Methylprednisolone acetate (Depo Medrol) 10–20 mg IM every 4–8 weeks
- Metered-dose inhaler (e.g., fluticasone at a dose of 44 μg per puff q 12 h).

β agonists (for acute treatment):

- Terbutaline
- Albuterol.

Methylxanthines:

- Theophylline
- Aminophylline.

Adverse effects in cats are a concern with long-term treatment (Box 48.3). Among the concerns are that corticosteroids may exacerbate or increase the risk of congestive heart failure in cats (Smith et al., 2004; Ployngam et al., 2006). The proposed mechanism for increasing this risk is increase in plasma glucose, volume expansion, and increase in plasma volume. The vascular volume increase may lead to an overload. To avoid systemic adverse effects, topical (inhaled) formulations have been used (see Section Inhaled Corticosteroids). In cats with feline asthma and risks such as heart disease or diabetes mellitus, cyclosporine may be considered as an alternative (Nafe and Leach, 2015). Use of cyclosporine in cats is discussed in more detail in Chapter 45.

Box 48.3 Possible adverse effects of corticosteroids in cats

- Behavior changes
- Increased appetite
- Weight gain
- Hyperglycemia, and increased risk of diabetes mellitus
- Increased plasma volume (secondary to hyperglycemia)
- Increased risk of heart disease (secondary to increase in plasma volume)
- Hepatomegaly

Horses

In horses, corticosteroids are important for management of recurrent airway obstruction (RAO) and inflammatory airway disease (IAD) in addition to environmental control and other measures (Box 48.1). Both prednisolone and dexamethasone, when combined with environmental control, had beneficial effects on heaves score, blood gases, and endoscopic scores (Courouce-Malblanc et al., 2008). Even with continuous antigen exposure, oral prednisolone and dexamethasone improved pulmonary function in horses with airway obstruction (Leclere et al., 2010). Inhaled corticosteroids delivered by metered-dose inhalers (discussed in Section Inhaled Corticosteroids) are more expensive but have been used to minimize the risk of systemic adverse effects.

Although oral corticosteroids are used in horses, there are no convenient dose formulations developed specifically for horses. In some cases tablets have been crushed and added to a drug vehicle or mixed with feed (grain). In horses, one should be cognizant of the dose form administered. Prednisone in horses is either not absorbed and/or not converted to the active prednisolone after absorption (Peroni et al., 2002). Therefore, prednisolone tablets are the preferred drug for oral administration. The dose used initially is approximately 2 mg/kg oral, once a day then tapered to 1 mg/kg. Oral dexamethasone is absorbed orally in horses and also can be used. It has been added to the horse's feed or mixed with a paste. The typical oral dose of dexamethasone is 0.1–0.05 mg/kg once per day then tapered to the lowest effective dose. In a study in which dexamethasone was effective for decreasing inflammatory cytokine expression in RAO horses, a decreasing oral dexamethasone dose of 0.165 mg/kg per day to 0.04 mg/kg per day over a course of 21 days was used (DeLuca et al., 2008).

Injections of dexamethasone have quickly improved clinical signs of airway obstruction when administered at doses of 0.1 mg/kg (Rush et al., 1998). Dexamethasone by injection was more effective than inhaled corticosteroids in that study. In other studies (Leclere et al., 2010; Courouce-Malblanc et al., 2008) dexamethasone was more effective than oral prednisolone for improving pulmonary function and bronchioalveolar lavage (BAL) cytology. An FDA-approved drug for horses is the suspension of dexamethasone 21-isonicotinate (Voren). It is administered at a dose of 0.06 mg/kg, and up to 0.1 mg/kg IM (not intended for IV use), then tapered to 0.02–0.05 mg/kg once daily, or every other day. Some of the cited studies demonstrate decreased inflammation in respiratory fluids. However, in one study, there was clinical improvement but no effect on BAL fluid cytology or NF-κB and AP-1 activity (Couëtil et al., 2006). Therefore, there may be improvements in pulmonary function in RAO, but are not always accompanied by a decrease in airway inflammatory cells or a suppression of inflammatory cytokines. Nevertheless, there is agreement among equine experts that the best results are obtained when corticosteroids administration is combined with environmental control (Couëtil et al., 2016; Ivester and Couëtil, 2014; Mazan, 2015). There is a concern that repeated use of injections of corticosteroids in horses may result in adverse effects, such as adrenal suppression, signs of hyperadrenocorticism, and laminitis, *although proof of corticosteroid-induced laminitis is inconclusive* (Robinson et al., 2009). Inhaled corticosteroids may minimize the risks of adverse effects (see Section Inhaled Corticosteroids).

Inhaled Corticosteroids

Glucocorticoids are among the most valuable drugs for managing asthma in people. For people, a metered-dose inhaler is used to deliver the drug topically in order to avoid systemic adverse effects (Derendorf et al., 2002). According to one review, inhaled corticosteroids are the most effective agents available for the symptomatic control of asthma and improvement in pulmonary function (Busse and Lemanske, 2001). Examples of aerosolized corticosteroids are listed in Table 48.4 and include beclomethasone (Beclovent®), flunisolide (Aerobid®), fluticasone (Flovent®), triamcinolone (Azmacort®), and budesonide (Pulmicort®). Fluticasone is the most potent (18 × dexamethasone) and is usually the one used in

Table 48.4 Examples of corticosteroids available as metered-dose inhalers

Drug	Brand name	Dose Delivered
Beclomethasone dipropionate	Vanceril®	40 or 80 µg per puff
Budesonide	Pulmicort®	200 µg per puff (dry powder)
Flunisolide	Aerobid®	250 µg per puff
Fluticasone propionate (most potent)	Flovent®	44, 110, or 220 µg per puff
Triamcinolone acetonide	Azmacort®	100 µg per puff

veterinary medicine (Table 48.4). In people it has a systemic absorption of only 18–26%, but there are extensive first-pass effects and high plasma protein binding preventing activity of systemic blood concentrations if it is swallowed after delivery. Therefore, the systemic action and adverse effects are minimized, even though some adrenal suppression can be detected. Suppression of the hypothalamic–pituitary–adrenal axis can still be observed in animal studies because this is a very sensitive measurement of systemic exposure.

Use in Horses

The metered-dose inhalers designed for people can be used in horses with the aid of an equine-adapted handheld metered-dose delivery device (Derksen et al., 1996; Rush et al., 2000). Delivery devices used in horses include the AeroHippus® mask (Trudell Medical International). One of the limitations for the use is the expense of the metered-dose inhaler products; however, when cost is not a factor these agents have been effective and the use supported by equine medicine experts (Couëtil et al., 2016; Ivester and Couëtil, 2014; Mazan, 2015; Pirie, 2014) (Box 48.1).

There is convincing evidence that aerosolized corticosteroids improve clinical signs and pulmonary function, but effects on other factors can be inconsistent and dependent on the study design and whether or not there was accompanying measures to control the environment. Aerosolized beclomethasone dipropionate using an equine-adapted metered dose inhalation was effective for reducing airway inflammation in horses with RAO (Rush et al., 1998, 2000). Horses responded within 24 hours. Administration of 500 μg every 12 hours to horses produced less adrenal suppression than larger doses and still provided beneficial effects. Administration of 500 μg of beclomethasone every 12 hours for 10 days improved pulmonary function in horses with RAO, but did not change bronchoalveolar lavage fluid cytology or cytokine activity (Couëtil et al., 2006). In this study, the effects of inhaled beclomethasone on pulmonary function were not accompanied by a decrease in airway inflammation or suppression of transcription factors NF-κB and AP-1.

Fluticasone also is frequently used (Table 48.4). Fluticasone treatment to horses with heaves produced resolution of clinical signs, normalization of pulmonary function, and significant decrease in BAL neutrophilia. There was also a significant decrease in inflammatory cytokine expression (Giguère et al., 2002). When administered at a dose of 6,000 μg every 12 hours it was as effective as dexamethasone for prevention of acute exacerbations of RAO, but not as effective for treatment as for prevention (Robinson et al., 2009). Fluticasone (2,000 μg twice daily initially then 2,000 μg once a day) produced significant improvement in clinical signs and lung function and no significant adverse effect on innate and acquired humoral and cell-mediated immune parameters (Dauvillier et al., 2011). Currently recommended doses are betamethasone 1–8 μg/kg every 12 hours or fluticasone 1–6 μg/kg every 12 hours (Couëtil et al., 2016).

Use in Small Animals

Metered-dose inhalers have also been successfully used in small animals, and are an accepted treatment for feline asthma (Trzil and Reinero, 2014; Reinero, 2011) (Box 48.2). For this use, the AeroKat® chamber described earlier has been used to deliver the drug. Budesonide or fluticasone are most often administered because of their high potency (Table 48.4) and low systemic effects. A typical dose for fluticasone for a cat is 110 μg (one puff from a 110 μg metered dose inhaler) per day. Higher-dose inhalers that deliver 220 μg are also available, but in one study in cats (Cohn et al., 2008) three fluticasone doses (44, 110, and 220 μg per cat) twice daily via metered dose inhaler were equivalent in an experimental model of feline asthma.

In one study, inhaled fluticasone reduced bronchial hyperresponsiveness and bronchoconstriction in cats with bronchitis at a dose of 250 μg per cat daily (Kirschuink et al., 2006). This dose also decreased inflammatory cells and prostaglandins in bronchoalveolar lavage fluid. When asthmatic cats are administered inhaled corticosteroids twice a day and allowed five to seven breaths (10 seconds) from the chamber, it reduces the need for oral prednisolone. In cats, flunisolide was studied for its systemic effects after administration by inhalation (Reinero et al., 2006). Although there was some suppression of the hypothalamic–pituitary–adrenal axis (indicating some systemic absorption), systemic effects on immune cells (lymphocytes and lymphocyte function) were not observed, which demonstrated that inhaled flunisolide is capable of producing a local effect in the airways, but with minimal effects on the systemic immune system.

Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) have been administered for some types of pulmonary disease. The drug most often investigated has been flunixin meglumine. It is registered for cattle to treat inflammatory respiratory problems and as an adjunct treatment of bovine respiratory disease (BRD) and is included in one of the antibiotic combinations used to treat BRD (flunixin meglumine and florfenicol in Resflor Gold®).

Diseases in which NSAIDs may have a benefit include pulmonary thromboembolism (for which antiplatelet doses of aspirin are used), pulmonary effects from endotoxin in horses and dogs (for which flunixin meglumine has been used), and in cattle with BRD, and pulmonary disease from PI-3 and 3-methyl indole in cattle.

Leukotriene Inhibitors

Leukotrienes, such as LTD_4 contribute to airway inflammation by increasing migration of eosinophils, producing bronchoconstriction, and increasing airway wall edema. Inhibitors or blockers of leukotrienes have been used to treat these airway diseases. These were discussed in more detail by Drazen et al. (1999) and reviewed by Werz and Steinhilber (2006) and Peters-Golden and Henderson (2007). The leukotrienes have not been demonstrated to be important mediators in feline asthma (Norris et al., 2003). Therefore, these inhibitors may not have a role in feline respiratory disease. However, there is evidence that LTB_4 – rather than the other leukotrienes – may contribute to airway inflammation in horses with recurrent airway obstruction (RAO) (Lindberg et al., 2004). An experimental 5-lipoxygenase inhibitor (fenleuton) – which decreases LTB_4– produced improvement in horses with RAO (Marr et al., 1998), but an LTD_4 receptor antagonist did not (Lavoie et al., 2002).

Lipooxygenase Inhibitor: Zileuton (Zyflo®)

Zileuton is an oral drug for treating asthma in people. It inhibits the enzyme 5-lipoxygenase and thereby inhibits synthesis of inflammatory leukotrienes. This drug has broader actions than the leukotriene receptor blockers because it inhibits effects of both LTB_4 and the cysteinyl leukotrienes (LTC_4, LTD_4, LTE_4). However, it has been less effective than leukotriene receptor blockers (see Section Leukotriene Receptor Blockers) because, at the doses administered, it does not achieve complete suppression of the 5-lipoxygenase enzyme. In people, the safety profile has not been as good as the receptor blockers. The effectiveness of zileuton for respiratory diseases in animals has not been reported. For people the dose is 600 mg, q 6 h and this dose has been extrapolated to animals for some studies. Treatment of other diseases in animals (e.g., allergic skin disease) have been investigated, but it has not been effective. In people it has been associated with hepatitis in some individuals.

Leukotriene Receptor Blockers

Zafirlukast (Accolate®), pranlukast, and montelukast (Singulair®) are leukotriene receptor blockers that have been used as oral drugs for treating asthma. They block the cysteinyl leukotriene receptor, therefore blocking the effects of LTC_4, LTD_4, and LTE_4– primarily at the $CysLT_1$ receptor site – but these drugs do not block receptors for the leukotriene LTB_4 (Drazen et al., 1999). The $CysLT_1$ receptor mediates sustained bronchoconstriction, mucus secretion, and edema in the airways (Peters-Golden and Henderson, 2007). These drugs have been generally well tolerated in people, but there are some drug interactions with zafirlukast (inhibition of CYP-450 enzymes). Montelukast has fewer adverse effects in people and less risk of drug interaction than zafirlukast. The use of these drugs has not been reported to be effective for treating respiratory diseases in animals. Doses in animals are not established, but the dose in people for zafirlukast is 20 mg/person twice daily and for montelukast it is 5 mg for children and 10 mg for adults.

Expectorants and Mucolytic Drugs

The expectorants and mucolytics comprise a diverse group of compounds, with many proposed benefits but few clinical studies that have documented efficacy. These drugs have been used to increase the output of bronchial secretions, enhance clearance of bronchial exudate, and promote a more productive cough. Some of these drugs have a traditional use in veterinary medicine but their efficacy has not been established.

Saline Expectorants

These drugs are promoted to stimulate bronchial mucus secretions via a vagal-mediated reflex action on the gastric mucosa. Even though there are no well-designed studies that support an expectorant effect from these products, particularly the ammonium compounds, stimulating the stomach appears to trigger a reflex that stimulates bronchial secretions. However, for some of these drugs, such as potassium iodide, the dose used clinically is too low to be effective. Examples of expectorants include ammonium chloride, ammonium carbonate, potassium iodide, calcium iodide, and ethylenediamine dihydroiodide (EDDI). EDDI is the best known of these compounds by veterinarians because it is also added to the feed of cattle for the purpose of decreasing foot rot infections, lumpy jaw (*Actinomyces bovis*), woody tongue (*Actinobacillus lignieresi*), and bronchitis. There is a lack of published scientific evidence for a beneficial effect.

Direct Stimulants

Volatile oils such as eucalyptus oil (found in cough drops such as Halls Mentho-Lyptus) and oil of lemon are believed to directly increase respiratory tract secretions. Their clinical efficacy in veterinary medicine is unknown.

Guaifenesin (Glyceryl Guaiacolate) and Guaiacol

Guaifenesin compounds are typically classified as muscle relaxants in anesthesia and as anesthetic adjuncts, but they also may have an expectorant effect. The anesthetic effects are discussed in Chapter 14. Their mechanism of action is uncertain, but it is possible that these compounds stimulate bronchial secretions via vagal pathways. Although guaifenesin is contained in many OTC cough remedies, such as Robitussin®, Vicks®, and others, the efficacy is highly questionable because most preparations do not contain a large enough dose. Studies that have measured the expectorant effect of guaifenesin have noted that it does not change the volume or viscosity of bronchial secretions, but it may accelerate particle clearance from the airways.

Formulations used in people (but not evaluated in animals) employ higher doses than older OTC drugs. The higher-dose formulations for people include Mucinex® (600 and 1200 mg tablet), Mucinex-D® (with pseudoephedrine), and Mucinex-DM® (with dextromethorphan). Most typical OTC older formulations contain doses of 100 mg.

Acetylcysteine (Mucomyst®)

Acetylcysteine is available as a 10% solution that can be nebulized to patients. It is used in human patients with obstructive airway disease and children with cystic fibrosis to help clear mucus. Its mucolytic effect is caused by an interaction of the exposed sulfhydral groups on the compound with disulfide bonds on mucoprotein (Ziment, 1988). Acetylcysteine helps break down respiratory mucus, reduce the viscosity of mucus, and enhance the clearance. Acetylcysteine may also increase the levels of glutathione, which is a scavenger of oxygen-derived free radicals. In Europe this drug is also available as an oral formulation. In human pediatric patients 2–4 ml of a 10% solution of *N*-acetylcysteine is nebulized three or four times daily. However, the use of nebulized acetylcysteine is discouraged in cats because it produced adverse effects and increased airway resistance (Reinero et al., 2011).

Other uses for acetylcysteine: Acetylcysteine may also increase the levels of glutathione, which is a scavenger of oxygen-derived free radicals. This property has led to the use of acetylcysteine to treat some intoxications. The most common condition is acetaminophen (Tylenol) toxicosis in cats.

Demulcents

Many OTC preparations are formulated in an oily or syrup base to provide a demulcent action (soothing effect). They are usually used to soothe the pharynx and thus may have an antitussive effect. Home remedies such as honey mixed with herbal tea or distilled spirits probably have this effect.

Decongestants

Decongestants are used to “dry up” mucus membranes when rhinorrhea occurs (in people, caused by the common cold and allergic rhinitis). Decongestants are sympathomimetic drugs that directly stimulate the α-adrenergic receptors, and indirectly by increasing release of norepinephrine. When α-receptors are stimulated in mucus membranes, a local vasoconstriction is produced which results in a decongestant effect. Alpha-receptors are also found in many tissues of the body, including, bladder sphincter, peripheral arteries and veins, coronary vessels, and myocardium. Therefore, stimulation of these receptors can lead to the adverse cardiovascular consequences. The pharmacology of adrenergic agonists was discussed in more detail in Chapter 7.

Topical Decongestants

Short-acting topical agents include phenylephrine and phenylpropanolamine, which are common ingredients in OTC nasal sprays (e.g., NeoSynephrine®). These products also have been applied topically to decrease bleeding associated with some surgical procedures (for example, nasal turbinate surgery in horses and dogs). Some topical decongestants are particularly long acting, such as oxymetazoline (Afrin®) and xylometazoline (Dristan®). Caution should be exercised when using the topical products chronically. Rebound inflammation and hyperemia may occur when the action of the drug diminishes, resulting in a worsening of the problem. The clinical use of these agents for nasal problems is rare in animals because they are difficult and inconvenient to administer.

Systemic Decongestants

The systemic use of adrenergic agonists as decongestants has been a common practice in medicine for decades. In human medicine, OTC (nonprescription) formulations for colds and allergies have contained ephedrine, the isomer pseudoephedrine (Sudafed®), or a similar drug, phenylpropanolamine (PPA). Side effects from the orally administered drugs include vasoconstriction and increased blood pressure in susceptible individuals, and excitement. A change in heart rate is possible from direct stimulation (increased heart rate), or vasoconstriction (decreased heart rate). In dogs, PPA toxicosis is possible either from accidental exposure or therapeutic doses. These effects were described in reports of exposure in dogs (Peterson et al., 2011). Clinical signs of toxicity in dogs consisted of cardiovascular effects (tachycardia, bradycardia, and hypertension), gastrointestinal signs (vomiting, salivation, and anorexia), and CNS effects (agitation, muscle tremors, and mydriasis). There are some problems with the abuse of these agents in humans, which has limited the veterinary availability of these compounds.

The original OTC forms of phenylpropanolamine are no longer used – popular brand names were Dexatrim® and Acutrim® (appetite suppressants), and Propagest® and Rhindecon ® (decongestants). A study in 2000 suggested that phenylpropanolamine in appetite suppressants, and possibly decongestants, is a risk factor for hemorrhagic stroke in women (Kernan et al., 2000). Because of the risk of hemorrhagic stroke in women, these products have been largely removed from the human market, but there are now veterinary forms of these drugs for treating urinary incontinence (see Section Use of Decongestants for Treatment of Incontinence).

The other major concern with OTC decongestants is the diversion to manufacturing methamphetamine. Phenylpropanolamine, ephedrine, and pseudoephedrine have been used as raw ingredients to illegally manufacture methamphetamine for “street use” in the so-called “meth labs.” Pseudoephedrine closely resembles methamphetamine in structure. Because of this diversion, the quantity of pseudoephedrine (the only one currently available OTC) that one can purchase is limited by most pharmacies. In some states, pseudoephedrine may be obtained without prescription, but it must be dispensed by the pharmacists (behind the counter). Pseudoephedrine has been eliminated from all OTC medications and replaced by less effective drugs.

Use of Decongestants for Treatment of Incontinence

Although these drugs are rarely used as sinus decongestants in veterinary medicine they are popular drugs for treating incontinence in dogs. The effect of phenylpropanolamine (PPA) and other sympathetic agonists, such as pseudoephedrine, for treating urinary incontinence arises from their stimulation of the α-adrenergic receptor – to increase the tone of the urinary sphincter, and its β-adrenergic receptor effects – to relax the detrussor muscle of the bladder wall, and allow more urine filling.

The most common drug used for this purpose has been PPA (Moreau and Lappin, 1989). PPA has been tested and is FDA approved for treating incontinence in dogs. The formulations of PPA marketed for treatment of urinary incontinence in dogs include tablets and liquid forms (e.g., Proin ®chewable tablets). The dose is approximately 2 mg/kg oral, q 12 h, but may be reduced to 1 mg/kg or administered less frequently in some dogs.

Respiratory Stimulants

Doxapram stimulates respiration by direct stimulation of the medullary respiratory center and activation of the aortic and carotid body chemoreceptors to improve sensitivity to carbon dioxide. These receptors are sensitive to changes in carbon dioxide, which in turn stimulate the respiratory center. Doxapram (Dopram®) is used primarily in emergencies, during anesthesia, or to decrease the respiratory depressant effects of certain drugs (e.g., opiates, barbiturates).

The primary use of doxapram in horses is for the treatment of respiratory acidosis in foals. The syndrome of hypoxic–ischemic encephalopathy (perinatal asphyxia or neonatal maladjustment syndrome) is common in neonatal foals. Doxapram has been administered as an initial IV dose of 0.5 mg/kg, followed by a constant rate infusion of 0.03 or 0.08 mg/kg/min for 20 minutes (Giguère et al., 2007). This administration has restored normal ventilation, improved blood pH, Pao_2, and respiratory rate in neonatal foals in a dose-dependent manner. In a clinical study by the same investigators (Giguère et al., 2008) a dose of 0.02–0.05 mg/kg/min constant rate infusion in foals with respiratory acidosis caused by neonatal maladjustment syndrome improved respiratory rate ventilation and $Paco_2$. Doxapram has also been administered to neonatal foals as a stimulant to increase their suckling activity shortly after birth and subsequently decrease the incidence of failure of passive transfer of immunoglobulins.

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49

Ophthalmic Pharmacology

Alison Clode

Pertinent Ocular Anatomy and Physiology

The ocular globe is housed within the orbit and protected by the bony and soft tissue components of the orbit, as well as the adnexal structures. The adnexal support tissues of the eye include the eyelids, nictitating membrane, conjunctiva, lacrimal glands, and nasolacrimal system, all of which serve various functions, such as mechanical protection (i.e., blinking and tearing from the eyelids), and immune surveillance (i.e., immune cells within the conjunctiva). The globe itself is composed of three tunics: the external, fibrous tunic (cornea and sclera), which provide rigidity as well as focusing ability; the middle vascular tunic (uveal tract = iris + ciliary body + choroid), which provide blood supply to the globe, maintain the intraocular pressure (IOP), and control light entry to the retina; and the neural tunic (sensory retina), which provides signal transmission requisite for visual function. The crystalline lens focuses light rays on the sensory retina, providing optimal focusing power for visual clarity. Aqueous humor (AH) produced by the ciliary body occupies the space between the cornea and the lens (anterior chamber), and nourishes the avascular and transparent cornea and lens. Vitreous humor (VH) occupies the space between the lens and sensory retina, and maintains structural integrity of the posterior portion of the globe. The globe may also be subdivided into the anterior segment (structures anterior to the junction of the retina and ciliary body), and posterior segment (structures posterior to this junction), a distinction important in consideration of ocular drug delivery and penetration.

Two blood–ocular barriers, the blood–aqueous and blood–retinal barriers, limit the entrance of blood components, such as large proteins, white blood cells, red blood cells, and lipid, into the eye, thus maintaining transparency of the ocular media necessary for vision. While effective at maintaining ocular clarity, these barriers also impede entrance of many drugs into the eye, thus potentially diminishing therapeutic effectiveness. Importantly however, intraocular inflammation, as is commonly present in ocular diseases necessitating pharmacotherapy, decreases effectiveness of the blood–ocular barriers, enabling some drugs to enter the eye.

Autonomic ocular innervation is both parasympathetic and sympathetic, with acetylcholine and norepinephrine, respectively, serving as the postganglionic neurotransmitters of the two systems. Most notable in the context of ocular pharmacotherapy is parasympathetic innervation of the lacrimal glands, iris sphincter muscle, and extraocular muscles, and sympathetic innervation of the adnexal and orbital smooth muscle and iris dilator muscle. Alpha- and beta-adrenergic receptors are also present on the ciliary body (where AH is produced) and iridocorneal angle (ICA) structures (where AH drains), thus impacting maintenance, and potential therapy, of IOP.

Factors Influencing Drug Levels in Ocular Tissues

Ocular Factors

Multiple barriers to drug penetration exist, which are inherent to ocular structures regardless of route by which drugs are administered.

Anteriorly, the lipophilic corneal epithelium is the major barrier to penetration of topically applied drugs. The underlying corneal stroma is hydrophilic, while the posteriorly located corneal endothelium is lipophilic. This lipophilic–hydrophilic–lipophilic layering limits intraocular movement of all but biphasic drugs, which have combined hydrophilic and lipophilic structural properties. Thus, treatment of disease conditions beyond the corneal barrier necessitates knowledge of drugs that effectively penetrate this barrier, either by their biphasic structure, or by susceptibility to alterations within the ocular environment itself. An example of medications altered by the ocular environment to improve penetration are the prostaglandin analogues, which are converted by esterases within the cornea, thus improving migration through the corneal layers.

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The blood–aqueous barrier and blood–retinal barriers are the major impediments to penetration of systemically administered drugs. Lipid-soluble, low-molecular-weight drugs are most able to penetrate intact (non-inflamed) barriers, while compromise of these barriers with inflammation increases the intraocular movement of larger, less lipid-soluble drugs, while generally maintaining the ability of lipid-soluble, low-molecular-weight drugs to penetrate.

The hyaloideocapsular ligament, located at the junction of the posterior lens capsule and anterior vitreous, is a barrier to the movement of drugs between the anterior and posterior segments of the eye. Disruption of this barrier, as may occur with intraocular surgery or lens luxation, increases posterior movement of topically applied drugs, and anterior movement of systemically administered drugs.

Drug Factors

Solubility impacts drug disposition within the eye, with biphasic and lipophilic drugs most readily able to penetrate the eye following administration topically and systemically, respectively. Additionally, low-molecular-weight drugs are more able to penetrate ocular barriers.

Administration of drugs formulated at physiological pH (~pH 7 to pH 7.4) best avoids discomfort upon instillation, thus improving drug availability, as irritation leads to tearing and subsequent drug washout. Drugs that require formulation at nonphysiological pH to maintain stability may be combined with buffers at the time of administration to improve comfort upon administration and subsequent retention on the ocular surface.

The formulation of topical medications also impacts drug disposition, and generally fall into the categories of solution, suspension, or ointment. Chapter 5 reviews the pharmaceutical science behind formulations. Solutions are generally easier to administer to small animals, and may be administered to large animals through subpalpebral lavage systems; however, spillage over the lids may occur with dosing quantities that are too large. The volume of drops from commercially manufactured dropper bottles ranges from 25 to 70 μl. As the palpebral fissure of dogs and cats holds only approximately 30 μl, administration of more than one drop at a time is likely to lead to overflow and spillage and subsequent loss of drug.

Suspensions are composed of larger particles suspended in an aqueous vehicle, and are administered from a dropper bottle. While generally minimally irritating, the particle size must be carefully controlled to avoid stimulating greater tear production and subsequent washout.

Ointments contain drugs within various viscous vehicles, such as petrolatum and lanolin. Advantages of ointments include the ability to administer lipid-soluble drugs, and to achieve a longer contact time with less drainage through the nasolacrimal system. A disadvantage of ointments, however, is the oily residue frequently remaining following administration.

Administration Factors

Medications to treat ocular disease are most commonly administered either topically or orally/parenterally, with local ocular injections (i.e., subconjunctival, intracameral, intravitreal) indicated in very specific circumstances.

Depending on specific drug factors (see Section Drug Factors), topical administration enables high levels of medication to be reached locally, specifically within the conjunctiva, cornea, and potentially the anterior chamber. Additionally, topical administration minimizes systemic exposure, thus minimizing the potential for adverse drug reactions or interactions. Systemic (oral, parenteral) medication is indicated for the treatment of eyelid and orbital disease, due to the restricted penetration of topical medications to these tissues, as well as extensive vascularization of the regions. Additionally, posterior segment disease is usually most effectively managed with systemic medications due to the presence of the corneal and hyaloideocapsular barriers anteriorly.

Subconjunctival administration involves the injection of medication into the space between the conjunctiva and the sclera. Such medications will then either penetrate the eye through the sclera and cornea or be absorbed by the conjunctival vasculature, thus increasing systemic exposure. Less commonly used, intracameral administration involves the injection of medication into the anterior chamber, allowing movement of drug within the intraocular environment and subsequent drainage from the eye with the AH. In addition to a brief residence time within the eye, intracameral injection carries a greater risk of both iatrogenic damage associated with the injection itself, and direct intraocular toxicity induced by drug on delicate intraocular structures. Intravitreal administration involves the injection of medication into the vitreous, which serves as a long-term reservoir of drug within the eye. These high and potentially sustained local drug levels increase the likelihood of both intraocular and systemic dissemination relative to that associated with topical administration. Peribulbar or retrobulbar injection involves injection of medication behind the globe, and is reserved for placement of local anesthetics around the globe to provide both akinesia and analgesia in association with ocular procedures. Regarding all four routes of ocular/periocular injection, each should be used sparingly (if at all) and only with detailed knowledge of potential side effects and toxicities (both ocular and systemic) of the specific drug being injected.

Table 49.1 Topically administered ocular hypotensives available for use in veterinary ophthalmology

Drug class	Representative agents	Suggested frequency	Contraindications
Beta-blockers	Timolol (0.25%, 0.5%) Betaxolol (0.25%, 0.5%)	q 12 h	Asthma Heart disease
Carbonic anhydrase inhibitors	Dorzolamide (2%) Brinzolamide (1%)	q 8–12 h	
Prostaglandin analogues	Latanoprost (0.005%) Bimatoprost (0.03%) Travoprost (0.004%)	q 12–24 h	Pupillary block glaucoma (uveitis, anterior lens luxation)
Parasympathomimetics	Pilocarpine (1–2%) Demecarium bromide (0.125–0.25%)	q 12–24 h	Pupillary block glaucoma (uveitis, anterior lens luxation)

In addition to the route of administration, frequency of administration may impact the success of therapy of ocular diseases. An advantage of topical administration is the ability to vary the frequency in association with the indication and the severity of the disease process. For example, prophylactic antimicrobial therapy of an uninfected corneal ulcer is appropriate and generally preferred to occur at a frequency of every 6 to 8 hours, while therapeutic administration of an infected corneal ulcer may be as frequent as every hour, thus potentially increasing the effectiveness of medical therapy.

Glaucoma

Physiology of Glaucoma

The intraocular pressure (IOP) is maintained by a balance between production and drainage of AH. Production of AH by the ciliary body occurs primarily via active secretion, utilizing both the carbonic anhydrase enzyme system and cAMP-mediated mechanisms. Following production posterior to the pupil, AH flows anteriorly through the pupil and drains out of the eye via conventional and unconventional outflow at the ICA. Conventional outflow through the trabecular meshwork into scleral vessels drains the majority of AH, while unconventional (uveoscleral) outflow drains the remainder (15% in dogs, 3% in cats, as much as 60–70% in horses).

Glaucoma is characterized by increased IOP, resulting in retinal and optic nerve damage and subsequent vision loss, as well as variable degrees of discomfort. Glaucoma is always caused by decreased drainage, either due to an inherent abnormality in the drainage angle itself (primary glaucoma) or obstruction of AH flow within or from the eye (secondary glaucoma).

Pharmacological Therapy of Glaucoma

Medical management of glaucoma is achieved utilizing medications that either decrease AH production or increase AH drainage (Table 49.1). It is important to note that, while drug studies frequently utilize normotensive eyes to determine IOP-lowering effects of individual drugs in various species, reductions achieved in glaucomatous eyes are frequently relatively greater.

Beta-blockers

Representative agents: 0.25%, 0.5% timolol (nonselective); 0.25%, 0.5% betaxolol (β_1-selective).

Mechanism of action: Beta-blockers decrease AH production by an unknown mechanism; however, interaction with either cAMP or Na^+/K^+-ATPase (or both) enzyme systems is speculated to be involved.

Dosing/efficacy: Beta-blockers are generally indicated for twice-daily topical ophthalmic dosing as maintenance (rather than emergency) therapy. Reduction of IOP by 4–5 mmHg is achieved in dogs with glaucoma, reduction of 4 mmHg is achieved in normotensive cats, and reduction of 7 mmHg is achieved in normotensive horses.

Side effects/contraindications: Beta-blockers are known to be absorbed systemically, and may induce bradycardia and systemic hypotension. They also may exacerbate asthma and compensated heart disease due to nonspecific beta-receptor blockade. It is therefore generally recommended to avoid use in small dogs and cats (i.e., <10 kg), particularly those with a history of cardiovascular disease.

Carbonic Anhydrase Inhibitors

Representative agents: 2% dorzolamide (topical); 1% brinzolamide (topical); methazolamide (oral); acetazolamide (oral).

Mechanism of action: Carbonic anhydrase inhibitors (CAIs) decrease AH production by blockade of the carbonic anhydrase enzyme system in the ciliary body epithelium. Conversion of CO_2 to HCO_3^- and subsequent movement of H_2O into the posterior chamber is thus inhibited, resulting in decreased IOP.

Dosing/efficacy: CAIs are generally indicated for three-times daily dosing as maintenance therapy, but may be used as frequently as every 15 to 30 minutes in the emergency management of acute glaucoma. Dorzolamide and brinzolamide have comparable efficacy in glaucomatous dogs, lowering IOP by 7–15 mmHg. Brinzolamide did not lower IOP in normotensive cats. Dorzolamide and brinzolamide lowered IOP by 2 and 5 mmHg, respectively, in normotensive horses.

Side effects/contraindications: Topical formulations may cause local ocular discomfort upon administration. Systemic administration may lead to metabolic acidosis due to diuretic effects. Systemic side effects generally do not develop with topical administration. Combined use of topical and systemic formulations has not been shown to be additive.

Prostaglandin Analogues

Representative agents: 0.005% latanoprost; 0.03% bimatoprost; 0.004% travoprost.

Mechanism of action: Prostaglandin (PG) analogues, which are chemically modified versions of the endogenous PG PGF_2, decrease IOP by increasing uveoscleral (nonconventional) AH outflow through activation of FP receptors. A marked rapid reduction in IOP is noted; however, the precise mechanism for the acute reduction in IOP is unknown. The long-term IOP reduction achieved with PG analogues is linked to structural alterations within the trabecular meshwork portion of the ICA. Current PG analogues are prodrugs, with the administered form of the drug converted by corneal esterases into the active form, thus increasing the local, rather than systemic, drug effects.

Dosing/efficacy: PG analogues are indicated for once- to twice-daily dosing as maintenance therapy in dogs with primary glaucoma, with twice-daily dosing producing more consistent IOP-lowering effects. Dosing may be as frequent as every 15 to 30 minutes when managing acute glaucoma in an emergency situation. Clinically significant IOP reductions of 20–60% result from latanoprost, travoprost, and bimatoprost administration to glaucomatous canine eyes. Current PG analogues are ineffective in cats due to receptor selectivity (ocular hypotensive effects in cats are mediated by EP receptors, which PG analogues do not possess affinity for). A clinically insignificant reduction in IOP of 1–3 mmHg is achieved following administration to normotensive horses.

Side effects/contraindications: Conjunctival hyperemia and moderate to marked miosis are noted in canine eyes. Due to the miotic effects, PG analogues are contraindicated in most cases of secondary glaucoma (i.e., glaucoma resulting from blockage of the pupil and subsequent obstruction of AH flow, which is exacerbated by miosis). Some discomfort may occur with instillation. Glaucomatous cats and horses are ineffectively managed with PG analogues, due to receptor specificity in cats and due to the significant inflammatory side effects (conjunctival hyperemia, miosis, flare) in horses.

Hyperosmotic agents

Representative agents: mannitol 20% (intravenous); glycerol (oral).

Mechanism of action: Administration of hyperosmotic agents causes the serum to become hyperosmotic to intraocular fluids, resulting in both fluid movement from the AH and VH, as well as a reduction in ultrafiltration of plasma (which contributes a small volume to AH production). Dehydration of the VH may also mechanically open the ICA by pulling the anterior vitreous face, lens, and iris leaflets posteriorly.

Dosing/efficacy: Mannitol is administered IV at a dose of 1 g/kg over 15–20 minutes, with coinciding water depravation for 4–6 hours postadministration, in the management of acute primary glaucoma only. Maximum IOP reduction of approximately 10 mmHg (normotensive dogs) is generally achieved within 2 hours. Glycerol is administered at a dose of 1–2 g/kg orally, and, in contrast to mannitol, may be administered as a component of long-term management.

Side effects/contraindications: The most significant ocular contraindication to the use of hyperosmotic agents is the presence of anterior uveitis, which is associated with leakage of blood constituents through the normally minimally permeable blood–aqueous barrier. Such leakage enables the hyperosmotic agent to leak in to the eye, resulting in an intraocular fluid shift and corresponding increase in IOP. Systemic contraindications to the use of hyperosmotic agents include cardiovascular disease (expansion of extracellular fluid volume will lead to exacerbation of cardiac failure), renal disease, and diabetes mellitus (glycerol is converted to glucose). Glycerol may also induce vomiting, particularly at higher doses. Due to systemic side effects and contraindications and the advent of more effective topical medications,

Table 49.2 Examples of topically administered corticosteroid formulations available for use in veterinary ophthalmology

Agent	Formulation	Indications	Contraindications
Prednisolone acetate (1%)	Suspension	Ocular surface inflammation Anterior intraocular inflammation	Corneal ulceration Nonulcerative corneal infection (stromal abscess)
Dexamethasone sodium phosphate (0.1%)	Solution Ointment	Ocular surface inflammation Anterior intraocular inflammation	Corneal ulceration Nonulcerative corneal infection (stromal abscess)
Hydrocortisone 0.5–1%	Solution Ointment	Ocular surface inflammation	Corneal ulceration Nonulcerative corneal infection (stromal abscess) Intraocular inflammation

use of hyperosmotic agents in veterinary ophthalmology is limited.

Parasympathomimetics

Representative agents: 1–2% pilocarpine (direct acting); 0.125%–0.25% demecarium bromide (indirect acting).

Mechanism of action: Parasympathomimemtics increase outflow by inducing miosis, thus widening the ICA and increasing AH outflow.

Dosing/efficacy: Pilocarpine is generally indicated for administration three to four times daily, and leads to 30–40% IOP reduction in glaucomatous dogs.

Side effects/contraindications: The acidic pH of pilocarpine solution causes irritation following topical administration. Due to the induction of miosis, pilocarpine is contraindicated in patients with uveitis and/or pupillary block glaucoma. The effect of parasympathomimetics on IOP is minimal compared to other classes of ocular hypotensive agents.

Ocular Inflammation and Antiinflammatory Therapy

Ocular inflammation is meant to serve a protective function; however, significant structural damage is induced by the inflammatory reaction, which in turn may have deleterious effects on vision and comfort. Antiinflammatory therapy is therefore essential to minimize secondary effects of inflammation, and to preserve ocular structure and function. Indications for therapy with topical antiinflammatories include the management of ocular surface inflammation (conjunctivitis, keratitis) and anterior segment inflammation (anterior uveitis). Systemic antiinflammatories are indicated for management of posterior uveitis.

Pharmacological Management of Intraocular Inflammation

Corticosteroids

Representative agents (Table 49.2)**:** 0.1% dexamethasone sodium phosphate; 1% prednisolone acetate; 1% hydrocortisone.

Mechanism of action: Corticosteroids bind to cellular glucocorticoid (GC) receptors, translocate to the nucleus, bind hormone responsive elements (HREs), and exert antiinflammatory effects through inhibition of the arachidonic acid cascade. These effects may be positive modulation via inducing expression of antiinflammatory proteins (i.e., lipocortin), or negative modulation via inhibiting expression of proinflammatory molecules (i.e., prostaglandins, cytokines). These mechanisms are more fully described in Chapter 29.

Dosing/efficacy: The location and severity of ocular inflammation determine both the frequency of administration and drug selection. Ocular surface inflammation may be effectively treated with topical hydrocortisone; however, hydrocortisone is ineffective for deep corneal or anterior uveal inflammation, due to its inability to penetrate the cornea. *Therefore, to effectively treat deep keratitis or anterior uveitis, a formulation, such as prednisolone acetate or dexamethasone alcohol, that effectively penetrates the cornea to reach the anterior segment is necessary.* Regardless of the location of inflammation, severely inflamed eyes may require every 2–4 hour dosing, while every 8–12 hour dosing may be indicated with lesser degrees of inflammation.

Side effects/contraindications: Long-term topical administration may result in the formation of corneal deposits

Table 49.3 Examples of topically administered nonsteroidal antiinflammatory formulations available for use in veterinary ophthalmology

Agent	Formulation	Indications	Contraindications
Flurbiprofen 0.03%	Solution	Ocular surface inflammation	Individual sensitivity
Diclofenac 0.1%	Solution	Anterior segment inflammation	Monitor with corneal ulceration
Ketorolac 0.5%	Solution		
Bromfenac 0.9%	Solution		
Nepafenac 0.1%	Solution		

(degeneration). Systemic absorption may occur with frequent and/or long-term administration, adversely affecting coinciding endocrine imbalances (such as diabetes mellitus, hyperadrenocorticism). *Topical corticosteroids are contraindicated in the presence of a corneal ulcer,* due to inhibited reepithelialization of the corneal wound and potentiation of ocular surface infections.

Nonsteroidal Antiinflammatory Agents

Representative agents (Table 49.3)**:** 0.03% flurbiprofen; 0.1% diclofenac; 0.5% ketorolac; 0.9% bromfenac; 0.1% nepafenac.

Mechanism of action: Nonsteroidal antiinflammatory agents (NSAIDs) exert antiinflammatory effects by inhibiting the production of PG through the constitutive cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2) enzyme systems. These mechanisms are fully described in Chapter 20 of this text.

Dosing/efficacy: As with corticosteroids, frequency of administration of topical NSAIDs depends upon the severity of the inflammation, with administration being as frequent as every 2–4 hours, or as infrequent as every 12–24 hours. NSAIDs are considered less effective than topical corticosteroids in the management of ocular inflammatory diseases. Transcorneal penetration of NSAIDs is variable; however, newer agents (bromfenac, nepafenac) are reported to have improved penetration (and therefore potentially improve therapeutic effect) relative to older NSAIDs, such as flurbiprofen.

Side effects/contraindications: While safer to use in the presence of corneal ulceration than topical corticosteroids, topical NSAIDs may inhibit reepithelialization of the cornea, and have been associated with acute corneal melts. Systemic uptake is less of a concern than with topical corticosteroids.

Ocular Infections and Antimicrobial Therapy

Antibacterial agents

As with antibacterial therapy for other body systems, ocular use may be either prophylactic or therapeutic. It is important to consider that, while certain gram-negative bacteria (such as *Pseudomonas aeruginosa*) are known to be potent ocular pathogens, the majority of ocular infections are caused by pathogenic effects of normal ocular surface bacteria, which are predominantly gram-positive. Therefore, when considering the appropriate antibiotic choice for prophylactic therapy, an agent with gram-positive activity is important.

The general principles of ocular antimicrobial therapy are:

1) Prophylactic use is always indicated for superficial, uncomplicated corneal ulcers.
2) Prophylactic use is indicated in association with adnexal surgical procedures in which the ocular surface (generally conjunctiva) is involved.
3) Therapeutic use is indicated for infected, complicated corneal diseases, such as ulcers and abscesses.
4) Therapeutic use is indicated for bacterial endophthalmitis.
5) When possible, cytology and culture/sensitivity results should guide antibiotic selection.
6) Ability of a specific antibiotic formulation to reach desired site of infection (deep cornea, anterior chamber) should guide antibiotic selection.
7) Side effects, both ocular and systemic, must be considered.

"Double Antibiotic" and "Triple Antibiotic" Preparations

Representative agents: neomycin, polymyxin B, gramicidin (solution); neomycin, polymyxin B, bacitracin (ointment); other combinations may be commercially available as well.

Spectrum: Gram-negative (neomycin, polymyxin B), and gram-positive (bacitracin, gramicidin) bacteria.

Indications/dosing: Due to broad-spectrum activity, combination products are useful for prophylaxis for ocular surface infections. Cases that have infection but have not been receiving a combination product may also be effectively managed. The lack of intraocular penetration limits usefulness in the presence of deep corneal infections or bacterial endophthalmitis. Dosing can be as frequent as hourly if therapeutic, or three times daily if prophylactic.

Side effects/contraindications: Hypersensitivity to neomycin may result in blepharoconjunctivitis. A potential (but rare) anaphylactic reaction to neomycin may occur in cats, thus use of combination products in cats is generally not advised.

Additional information: A common antibiotic–corticosteroid combination product, neomycin/polymyxin B/dexamethasone, is useful for treatment of ocular surface inflammation and secondary bacterial infection. It is critical to ensure, however, that such products are avoided when a corneal ulcer is present, due to the corticosteroid component. Proper education of staff and storage of products with and without corticosteroids in separate areas is encouraged.

Aminoglycosides

Representative agents: 0.3% tobramycin; 0.3% gentamicin (also, neomycin as component of above preparations).

Spectrum: Predominantly gram-negative, with gram-positive restricted to *Staphylococcus aureus.* There is good efficacy versus *Pseudomonas aeruginosa*, although increasing resistance has been reported. Due to inherent resistance, aminoglycosides are inactive versus anaerobes. See Chapter 35 for further discussion.

Indications/dosing: Used as prophylaxis or therapy for ocular surface infections, aminoglycosides have variable intraocular penetration, which limits use in patients with deep corneal infections or bacterial endophthalmitis. Dosing can be as frequent as hourly if therapeutic, or three times daily if prophylactic.

Side effects/contraindications: Of the aminoglycosides (and topical antibiotics in general), gentamicin demonstrates comparatively high toxicity to the epithelium. It must be considered, therefore, that its use in the presence of corneal ulcerations may be associated with decreased wound healing. In cases with documented infection and appropriate susceptibility, use of gentamicin is warranted.

Tetracyclines

Representative agent: 0.5% oxytetracycline (formulated with polymyxin B).

Spectrum: Good activity versus intracellular organisms, with poor efficacy versus *Pseudomonas aeruginosa, Staphylococcus* spp., and *Streptococcus* spp. See Chapter 34 for further discussion.

Indications/dosing: Oxytetracycline is useful for prophylaxis or therapy for ocular surface infections, particularly in cats. As a tetracycline, antimatrix metalloproteinase activity may improve keratomalacia. In addition, tetracyclines have demonstrated increased corneal epithelial wound healing, which is presumed to be in association with modulation of transforming growth factor beta (TGF-β) activity. Dosing for prophylaxis is generally three times daily, and for therapy can be as frequent as hourly.

Side effects/contraindications: Local ocular irritation may occur.

Fluoroquinolones

Representative agents: 0.3% ofloxacin; 0.3% ciprofloxacin (solution, ointment); 0.5% levofloxacin; 0.5% moxifloxacin; 0.3% gatifloxacin; 0.6% besifloxacin.

Spectrum: Older agents (ofloxacin, ciprofloxacin, levofloxacin) are more effective versus gram-negative bacteria, while newer agents (moxifloxacin, gatifloxacin) have increased gram-positive efficacy. See Chapter 37 for further discussion.

Indications/dosing: Older agents may be appropriately used for prophylaxis or therapy of ocular surface and intraocular infections. Ofloxacin demonstrates greater corneal penetration than ciprofloxacin, therefore may be more appropriately used in cases with deep corneal or intraocular infections. Newer agents are indicated for therapeutic use only, in patients with known ocular surface or intraocular infection. Dosing can be as frequent as hourly if therapeutic, or three times daily if prophylactic.

Side effects/contraindications: Fluoroquinolones may induce drug precipitation within cornea. Local irritation is rare.

Other Topical Ophthalmic Antibacterials

Chloramphenicol (1% ointment)

Spectrum: Gram-positive and gram-negative bacteria, and intracellular organisms.

Indications/dosing: Prophylaxis or therapy of ocular surface infections is provided by administration two or more times daily.

Side effects/contraindications: Fluoroquinolones *may* be associated with dose-responsive bone marrow suppression (reversible upon discontinuation of medication). Fluoroquinolones *may* be associated with idiosyncratic aplastic anemia (which is irreversible, and generally occurs after drug administration has already been discontinued).

Erythromycin (0.5% ointment)

Spectrum: Gram-positive bacteria, *Chlamydophila felis*, *Mycoplasma* spp.

Indications/dosing: Prophylaxis or therapy of ocular surface infections is provided by administration two or more times daily.

Side effects/contraindications: Local ocular irritation may occur; however, this is generally mild.

Antifungal agents

The most frequent indication for antifungal agents in veterinary ophthalmology is in the treatment of fungal infections of the cornea (keratomycosis) in horses. Such infections are common in warm, humid climates, and are most commonly caused by filamentous organisms such as *Aspergillus* spp. and *Fusarium* spp. See Chapter 38 for further discussion of antifungal drugs.

Polyenes

Representative agents: 5% natamycin; amphotericin B.

Mechanism of action: Polyenes bind ergosterol in the fungal cell membrane, forming a polyene–sterol complex. This complex induces membrane permeability, allowing leakage of intracellular nutrients and resulting in cell lysis. Depending on the concentration achieved at the site of infection and organism susceptibility, action may be either fungistatic or fungicidal.

Dosing: Both agents are considered broad spectrum; however, activity versus *Aspergillus* spp. may be weaker than versus other filamentous organisms. Polyenes have poor penetration following topical administration to a cornea with an intact epithelium, thus natamycin is generally administered in cases of confirmed ulcerative keratomycosis, as frequently as hourly in initial stages of treatment. Amphotericin B has anecdotally been administered subconjunctivally to minimize irritation associated with frequent topical administration; however, this should be performed under the guidance of a veterinary ophthalmologist.

Side effects/contraindications: Local ocular irritation is the primary side effect. Systemic administration of amphotericin B may induce nephrotoxicity. Concern of systemic uptake associated with subconjunctival administration is minimal due to the small dose relative to body weight.

Azoles

Representative agents: 1% voriconazole (formulation for IV injection); 1% miconazole (compounded); 1% itraconazole/30% dimethyl sulfoxide (DMSO) (compounded); ketoconazole (oral); fluconazole (oral).

Mechanism of action: Azoles inhibit the synthesis of ergosterol in the fungal cell membrane, increasing membrane permeability and leading to cell lysis. Additionally, inhibition of intracellular enzyme activity leads to accumulation of toxic intracellular metabolites.

Indications/dosing: Azoles are broad spectrum versus filamentous fungi, and are generally better versus *Aspergillus* spp. than *Fusarium* spp. Administration of topical agents is indicated in cases of ulcerative and nonulcerative keratomycosis, as frequently as hourly in initial stages of therapy. Although less important in ocular surface therapy than topical administration, oral antifungal agents may be indicated in cases of keratomycosis. In horses with keratomycosis, fluconazole may be administered orally (14 mg/kg once, then 5 mg/kg orally every 24 hours).

Side effects/contraindications: Topical ocular irritation is generally minimal. Monitoring liver enzymes may be indicated in animals receiving oral azoles. Oral fluconazole has been implicated in prolonged recovery from anesthesia in horses, possibly due to interactions with midazolam and ketamine.

Antiviral Agents

The most common indication for antiviral agents in veterinary ophthalmology is the treatment of feline herpesvirus-1 infections, which manifest as ocular surface disease in cats. Dogs and horses also may experience herpetic ocular surface disease; however, investigations into efficacy of available antiviral agents towards canine and equine herpesvirus variants are fewer than those involving feline herpesvirus-1. See Chapter 38 for further discussion of antiviral drugs.

Pyrimidine Analogues

Representative agents: 1% trifluridine; 0.1–2.5% idoxuridine (compounded).

Mechanism of action: Pyrimidine analogues are selectively incorporated into viral DNA in the place of thymidine, disrupting viral DNA synthesis.

Indications/dosing: As pyrimidine analogues exert their action on DNA synthesis, they are only active when virus is actively replicating. In association with the cytopathic viral effect, active viral replication is indicated by the presence of corneal and/or conjunctival ulceration. As they are virustatic rather than virucidal, frequent administration (five to eight times/day) in the presence of ocular surface ulceration is necessary.

Side effects/contraindications: The most commonly reported side effect is topical ocular surface irritation.

Purine Analogues

Representative agents: 1–3% vidarabine; 0.5% cidofovir; famciclovir (oral); (*acyclovir – see Side effects/contraindications*; *valacyclovir – see Side effects/contraindications*).

Mechanism of action: Purine analogues undergo an initial phosphorylation step by viral thymidine kinases, with subsequent intracellular transformations resulting in inhibition of viral DNA synthesis.

Indications/dosing: While still virustatic rather than virucidal, cidofovir has the advantage of necessitating only twice daily topical dosing, while vidarabine is generally dosed topically similarly to the pyrimidine analogues (five to eight times/day). The appropriate oral dose has not been determined for famciclovir at this time; however, doses of 62.5 mg once to twice daily have been anecdotally reported as efficacious in the treatment of FHV-1-mediated disease, while doses as high as 90 mg/kg three times daily have been evaluated as safe in experimental situations.

Side effects/contraindications: Topical ocular irritation may occur with topical administration. Nasolacrimal punctal occlusion has been reported in conjunction with cidofovir administration in people. *Acyclovir and valacyclovir should not be administered to cats (orally or topically) due to life-threatening toxicities (kidney, bone marrow).*

Lacrimogenics

Lacrimogenics are drugs that increase tear production in cases of immune-mediated and neurogenic keratoconjunctivitis sicca (KCS).

T-cell inhibitors

Representative agents: 0.2–2% cyclosporine (commercially available, compounded); 0.03% tacrolimus (compounded).

Mechanism of action: Calcineurin inhibitors bind cyclophilin (cyclosporine) or FK-binding proteins (tacrolimus), subsequently inhibiting T-lymphocyte proliferation.

Indications/dosing: Topical administration is only appropriate for ocular surface disease (i.e., KCS, pigmentary keratitis, superficial keratitis), due to insufficient intraocular penetration for treatment of intraocular diseases (i.e., anterior uveitis). Treatment is generally twice daily, allowing a minimum of 4 weeks of therapy to determine response. Systemic administration may be utilized in the treatment of immune-mediated inflammatory diseases such as uveodermatological syndrome. Sustained-release, surgically implanted subconjunctival cyclosporin implants have been developed as treatment for Immune-Mediated Keratitis in horses and have been used with success as treatment for KCS in dogs that are not amenable to daily topical treatment. Sustained-release, surgically implanted suprachoroidal cyclosporine implants have been developed for treatment of Equine Recurrent Uveitis as well, but these are not indicated for treatment of ocular surface disease. Placement of these implants is best performed by a veterinary ophthalmologist with microsurgical training.

Side effects/contraindications: Topical administration of cyclosporine has resulted in suppression of cellular immunity in dogs; however, the clinical significance appears to be minimal. The effect of topical cyclosporine on viral (herpetic) keratitis is unknown.

Parasympathomimetic

Representative agent: 2% pilocarpine.

Mechanism of action: As a direct-acting parasympathomimetic, pilocarpine mimics acetylcholine at the terminal synapse. Dogs with neurogenic KCS are lacking neurological input to an otherwise normal lacrimal gland, thus pilocarpine bypasses the interrupted neurological pathways and provides direct stimulation to the lacrimal gland. See Chapter 8 for further discussion of the mechanism of action of this drug.

Indications/dosing: Oral administration of the 2% ophthalmic solution is utilized, generally starting as 1 to 2 drops per 20 pounds twice daily, applied on the food, and increasing until tear production is achieved.

Side effects/contraindications: Adverse systemic effects may be noted as dosing increases, resulting in signs of parasympathetic overstimulation and excessive salivation, lacrimation, urination, and defecation. When these signs are present, administration must be decreased.

Mydriatics and Cycloplegics

Mydriatics (drugs that dilate the pupil) are indicated primarily for diagnostic examination, while those with coinciding cycloplegic action that paralyze ciliary body muscle spasm are indicated for therapy of anterior uveitis.

Sympathomimetics

Representative agents: 0.1%, 1%, 2% epinephrine; dipivalyl epinephrine; 2.5%, 10% phenylephrine.

Mechanism of action: Sympathomimetics directly stimulate α-adrenergic receptors of the iris dilator muscle, producing mydriasis without cycloplegia. See Chapter 7 for further discussion.

Indication: Sympathomimetics are used to augment mydriasis achieved by parasympatholytics prior to intraocular surgery, and to diagnose Horner's syndrome.

Dosing/efficacy: Use of sympathomimetics when indicated is generally restricted to one to two drops topically, without repeated dosing. Their efficacy as mydriatic agents is less than that of parasympatholytic agents, thus they are generally not used alone for either diagnostic or therapeutic purposes.

Side effects: Ocular surface irritation may occur following topical administration. Systemic absorption may induce systemic hypertension, thus use of these agents should be limited in patients with preexisting cardiovascular disease, or in small (i.e., <10 kg) patients.

Parasympatholytics

Representative agents: 0.5%, 1% tropicamide; 1% atropine.

Mechanism of action: Parasympatholytics induce anticholinergic blockade of iris sphincter and ciliary body muscles, thus inducing mydriasis while producing cycloplegia. Additionally, stabilization of a leaking blood–aqueous barrier is achieved with atropine, thus partially mitigating the increased permeability associated with inflammation. Inflammation decreases the efficacy of parasympatholytics; however, due to stimulation of acetylcholine receptors on the iris sphincter muscles, higher drug concentrations are required to achieve pharmacological blockade. Mydriasis achieved with parasympatholytics is greater than that achieved with sympathomimetics, as the iris sphincter muscle is more powerful than the iris dilator muscle.

Dosing/efficacy: Dosing of parasympatholytics for diagnostic evaluation (tropicamide) is one to two drops topically once at the time of examination, with mydriatic effects generally wearing off within 6–8 hours. Dosing of parasympatholytics in the treatment of anterior uveitis (atropine) is to effect, as gauged by the induction of mydriasis. Depending on the degree of intraocular inflammation, one drop generally every 12–48 hours is appropriate. In addition to being influenced by disease process, relative efficacy is also dependent upon species; for example, mydriasis due to atropine lasts 4–5 days in normal canine eyes, versus up to 14 days in normal equine eyes. Relative efficacy also depends upon iridal pigmentation, as pigment serves as a reservoir for drug, slowing onset of action and prolonging duration in heavily pigmented versus poorly pigmented eyes.

Side effects/contraindications: Salivation may be induced following topical administration due to drainage of bitter-tasting atropine solution into the oral cavity via the nasopharynx. Temporary reduction in aqueous tear film production occurs. Photophobia may occur due to mydriasis.

Local Anesthetics

Topical local anesthetics are frequently administered to provide corneal and conjunctival anesthesia when performing routine diagnostic and therapeutic procedures, such as tonometry, cytology collection, and corneal or intraocular surgery.

Representative agents: 0.5% proparacaine; 0.5%, 1% tetracaine.

Mechanism of action: Local anesthetics interfere with nerve cell sodium channels, blocking the intracellular movement of sodium into nerve cells and thus inhibiting depolarization. See Chapter 15 for further discussion on local anesthetic mechanism of action.

Dosing/efficacy: Following administration of a single drop in dogs, anesthetic effect of proparacaine is noted within 1 minute, reaching maximal effect within 15 minutes, for a total duration of effect of 45 minutes. A second drop administered 5 minutes after the first can extend the duration of action to 55 minutes. In both cats and horses, a single drop of proparacaine results in anesthetic effect within 1 minute and lasts 25 minutes. Tetracaine administration in horses has a dose-dependent effect, with the 1% concentration exhibiting longer duration of anesthetic effect than the 0.5% solution (50 versus 30 minutes, respectively).

Side effects/contraindications: Proparacaine causes transient tear film instability, and repeated dosing may result in punctate epithelial keratitis, necrotizing keratitis, or severe infiltrative keratitis. Therefore, topical local anesthetics are *absolutely contraindicated* as therapy for any ocular surface disease, and repeated dosing should be performed with caution in a diagnostic setting. Topical local anesthetics may exert antimicrobial effects, thus samples for culture are ideally collected prior to administration. This may not be feasible in a clinical setting, however, due to the need to minimize patient discomfort in order to maximize patient cooperation.

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Pharmacogenomics

Katrina L. Mealey

A patient's response to a drug includes both beneficial (therapeutic) effects as well as any adverse effects, including lack of therapeutic efficacy. While the goal of drug therapy is to produce a specific pharmacological effect without producing adverse effects, it is often difficult to predict how effective or how safe a medication may be for a particular patient. If 10 patients with the same disease were administered the same drug, each might respond differently with respect to both drug efficacy and the likelihood of an adverse reaction. A number of factors may influence a patient's response to drug therapy, including the patient's age, species, concurrent medications, diet, health or disease status, and others. Many of these are discussed in Chapter 2 of this text. However, consideration of all of these factors is often not sufficient to explain the degree of interpatient variation observed. The observed interpatient variability in drug response may result from genetically determined differences in drug metabolism, drug distribution, and/or receptors or drug target proteins. The branch of pharmacology that involves identifying genetic variations leading to interindividual differences in drug response is called *pharmacogenetics*. Pharmacogenetics is likely the ultimate way to establish the right drug and dose for each patient, thereby optimizing efficacy and minimizing toxicity. Despite the fact that this branch of pharmacology is still in its infancy as a science, a number of important discoveries have already contributed to improved pharmacotherapy in human and veterinary patients.

The term *pharmacogenetics* is often used interchangeably with the term *pharmacogenomics*. Strictly speaking, pharmacogenetics refers to monogenetic (single gene) variants that affect a patient's response to a particular drug. An example of a pharmacogenetic study might be one that examines the influence of mutations in the thiopurine methyl transferase gene on the response to azathioprine. Pharmacogenomics, on the other hand, refers to the entire spectrum of genes that are involved in determining a patient's response to a particular drug. An example of a pharmacogenomic study would be one that examines the interaction between cytochrome P450 genes, β_1, β_2, α_1, and α_2-adrenergic receptor genes, and drug transporter (i.e., *MDR1*) genes on the response to carvedilol. The relatively few reports of gene–drug responses in veterinary medicine are pharmacogenetic in nature. While dramatic alterations in drug response have been identified in pharmacogenetic studies, a patient's overall response to a particular drug will likely require a pharmacogenomics approach to characterize (and summate) the individual contribution of multiple genes to drug response. For the purposes of this chapter, the terms *pharmacogenetics* and *pharmacogenomics* will be used interchangeably.

The concept that inheritance might explain individual variation in susceptibility to adverse drug reactions and/or variation in drug efficacy was proposed in 1957. The term *pharmacogenetics* was coined shortly afterward, in 1959. Relatively little pharmacogenetic research occurred for the next three decades. A resurgence in pharmacogenetic research occurred coincidentally with the initiation of the Human Genome Project in 1990. Since then, research in pharmacogenetics has expanded at a remarkable rate. There are now at least five journals exclusively devoted to pharmacogenetic/pharmacogenomic research, and many other medical and pharmacological journals encouraging submissions reporting results of pharmacogenetic research. Following completion of the Human Genome Project (announced in 2003), the National Human Genome Research Institute challenged investigators to develop genome-based approaches to predict drug response, setting the stage for continued growth in the field of pharmacogenetics.

Basic Genetic Concepts

The human genome contains approximately 3 billion nucleotide bases, representing roughly 30,000 genes. When a gene is expressed, DNA is transcribed into RNA, which is then translated to make proteins. Three consecutive nucleotide bases form a specific codon,

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specifying the addition of a particular amino acid or signaling amino acid chain termination (stop codons). The genetic code is said to have redundancy, which simply means that there may be two or more different codons for the same amino acid. In humans, for example, both GGA and GGC code for the amino acid glycine. Redundancy exists in the canine genome as well, with TAA, TGA, and TAG all representing stop codons. A gene is simply the DNA sequence containing a series of codons that specify a particular protein. The variation that occurs between individuals in a population is a result sequence differences in specific genes. These sequence differences are often the result of mutations.

A mutation alters the sequence of nucleotide bases in a DNA molecule. This in turn alters the transcribed RNA, creating a different codon. Some mutations, because of the redundancy described above, are silent, meaning that if the mutation results in a base change that creates a codon for the same amino acid (i.e., GGA to GGC) as specified by the original DNA sequence, there is no change in protein structure or function. However, if the mutation results in a different amino acid, or the creation of a stop codon, the change in protein structure and function can be deleterious. At each gene locus, an individual carries two alleles, one from each parent. An allele is defined as the DNA sequence at a given gene's location on the chromosome. If an individual has two identical alleles, that individual is said to have a homozygous genotype. If an individual has two different alleles, that individual is said to have a heterozygous genotype. The phenotype of each individual with regard to a specific gene is the outward, physical manifestation of a given genotype. That outward physical manifestation might be something immediately obvious in a given individual such as eye color, or it may not be apparent until a particular drug is administered to that individual.

Variations in a given gene may be present rarely in a population, or in relatively large numbers in a population. Polymorphisms are defined as genetic variations occurring at a frequency of 1% or greater in the population (species of interest). In humans, many of the genes encoding cytochrome P450 enzymes are polymorphic (specific mutations are present in greater than 1% of the population), whereas some inherited human diseases such as cystic fibrosis are caused by rare mutations occurring in less than 1% of the population. For some diseases, identification of a specific mutation may be used to provide specific treatment approaches for the patient and, in the case of veterinary patients, guide breeding decisions as well. However, many common diseases in human patients, such as diabetes mellitus and many cardiovascular disease, are polygenic (more than one gene contributes to the disease). For diseases that are polygenic in nature, the pathophysiology of the disease is complex, and specific treatments based on particular mutations will be difficult to sort out. It is likely that many important diseases in veterinary medicine (i.e., hip dysplasia, epilepsy, most types of cancer) are polygenic also.

Pharmacogenetics

Genetic variation can affect both the pharmacokinetics (i.e., drug absorption, distribution, metabolism, and excretion) and pharmacodynamics (i.e., interaction with drug transporters and receptors) of pharmaceutical agents. The concept of pharmacogenetics originated in the 1950s when it was discovered that the antimalarial drug primaquine caused hemolysis in a subpopulation of individuals (Hochstein, 1971). Ultimately, it was shown that the enzyme glucose 6-phosphate dehydrogenase had reduced function in affected individuals as compared to the majority of the population (Hochstein, 1971). At the time, techniques to study molecular biology were not available, so the field of pharmacogenetics was initially based purely on phenotypic observations (measurement of enzyme function). Discovery of the specific mutation in the glucose 6-phosphate dehydrogenase gene would not occur until a few decades later.

With the rapid advancement of molecular techniques, modern pharmacogenetic research differs significantly from those initial phenotypic observations. It currently involves identifying both the phenotype and the genetic variation responsible for it. Researchers perform systematic searches to identify functionally significant variations in DNA sequences in genes that affect drug disposition. In many instances, the genetic variation in a gene is identified before the phenotypic consequence is known. The availability of published sequences for the canine genome, feline genome, and genomes of other veterinary species has accelerated the progress of pharmacogenetic discoveries, facilitating the ultimate goal of pharmacogenetics, which is individualization of drug therapy.

It is important to note that individualization of drug therapy encompasses two distinct, yet equally important, clinical implications. First is the ability to predict those patients at high risk for developing drug toxicity. These patients may have a mutation in a drug-metabolizing enzyme that decreases drug clearance. For such patients a lower drug dose, longer dosing interval, or alternate drug should be administered. Second is the ability to predict those patients that are most likely to benefit from a particular drug because of appropriate receptor interactions. Patients with mutations in drug receptors may be poor responders to certain pharmaceutical agents. Rather than using a trial-and-error approach to drug therapy, a veterinarian could select the drug most likely to produce the desired pharmacological response in a particular patient, decreasing the amount of time in which the patient's disease state is poorly controlled.

Described in this chapter are several recent discoveries in veterinary pharmacogenetics and examples of pharmacogenetically based differences in drug absorption, distribution, metabolism, excretion, and drug–receptor interactions. The fundamentals of these topics were extensively discussed in Chapters 2, 3 and 4. The impact of these discoveries in clinical veterinary medicine will also be presented.

Pharmacogenetics of Oral Drug Absorption

Systemic bioavailability of orally administered drugs is often considered to be a function of physicochemical characteristics of the drug and subsequent hepatic metabolism. A number of other factors have recently been shown to impact the ability of a drug to be absorbed into the systemic circulation after oral administration. Intestinal Phase I drug metabolism and active drug extrusion by efflux transporters are now considered to be among the most important determinants of oral drug bioavailability. Consequently, genetic variation in intestinal drug metabolizing enzymes and drug transporters as well as hepatic drug metabolizing enzymes and transporters is likely to dramatically affect oral drug absorption.

In people, CYP3A is expressed at higher levels in mature villus tip enterocytes than in hepatocytes (Patel and Mitra, 2001). Because intestinal villi comprise such a large surface area, there is a high likelihood that absorbed drug will interact with intestinal CYP3A enzyme, facilitating substantial first-pass metabolism. Interpatient variability in intestinal CYP3A levels has been studied in a small sample of human patients. Elevenfold variations in CYP3A protein content and sixfold variation in enzymatic activity were identified, suggesting that CYP3A polymorphisms exist in the human population (Scordo and Spina, 2002). While intestinal drug metabolism is thought to be important in veterinary patients also, relatively little is known with regard to interpatient variability in enzyme activity.

Drug transporters are also known to play an important role in drug absorption. Many drug transporters have been identified in people, but the most well-characterized drug transporter is P-glycoprotein (P-gp), the product of the *MDR1* (also known as the *ABCB1*) gene. The potential impact of transporter pharmacogenetics on drug pharmacokinetics is dramatically illustrated by P-gp. P-gp is a transmembrane protein that was first described in highly resistant tumor cell lines (Roninson, 1992). Tumor cells expressing P-gp were cross-resistant to various anticancer agents (anthracyclines, vinca alkaloids, taxanes, and others). P-gp has since been shown to act as an ATP-dependent pump that exports drugs from nonneoplastic cells as well. In normal mammalian tissues, P-gp appears to function in a protective capacity. P-gp is expressed on bile canaliculi, renal tubular epithelial cells, the placenta, brain capillary endothelial cells, and at the luminal border of intestinal epithelial cells (Thiebaut et al., 1987). At these locations, P-gp pumps selected drugs either out of the body (into the bile, urine, or intestinal lumen) or away from protected sites (brain tissue, fetus).

The significant role intestinal P-gp can play in determining oral drug bioavailability has been demonstrated in rodent studies. In *mdr1* (−/−) knockout mice, oral bioavailability of many P-gp substrate drugs (vinblastine, taxol, digoxin, loperamide, ivermectin, cyclosporine A, and others) is substantially greater than in wild-type mice (Schinkel et al., 1995; Sills et al., 2002). Similarly, *MDR1* polymorphisms in people have been shown to result in altered oral bioavailability of P-gp substrate drugs. Studies have shown that oral bioavailability of digoxin, a P-gp substrate, is greater in subjects with the *MDR1* 3435TT genotype compared with those with the *MDR1* 3435CC genotype (Verstuyft et al., 2003). The P-gp substrate phenytoin has also been shown to have lower oral bioavailability in subjects with the *MDR1* 3435CC genotype (Kerb et al., 2001).

P-gp has been fairly well characterized in dogs. The tissue distribution of P-gp in dogs is similar to that in people (Ginn, 1996), and it has been shown to contribute to chemotherapeutic drug resistance in vitro and in vivo (Mealey et al., 1998; Page et al., 2000; McEntee et al., 2003). Its role in determining oral drug bioavailability in dogs has been characterized only indirectly until recently. The indirect evidence to support that P-gp is important in oral drug absorption in dogs was generated from studies using P-gp inhibitors. Bioavailability of the anticancer agent (and P-gp substrate) docetaxel was increased 17-fold when coadministered with a P-gp inhibitor (McEntee et al., 2003).

More recently, P-gp's role in oral drug absorption was investigated in dogs affected by an important polymorphism of the *MDR1* (*ABCB1*) gene. This polymorphism consists of a four-base-pair deletion mutation (*ABCB1-1*Δ). This deletion results in a shift of the reading frame that generates several premature stop codons (Mealey et al., 2001). Because protein synthesis is terminated before even 10% of the protein product is synthesized, dogs with two mutant alleles exhibit a P-gp null phenotype, similar to *mdr1* (−/−) knockout mice. Affected dogs include many herding breeds. For example, roughly 75% of collies in the United States, France, and Australia have at least one mutant allele (Mealey et al., 2002). Other affected herding breeds, albeit at a lower frequency, include Old English Sheepdogs, Australian Shepherds, Shelties, English Shepherds, Border Collies, German Shepherds, Silken Windhounds, McNabs, and Long-haired Whippets (Neff et al., 2004).

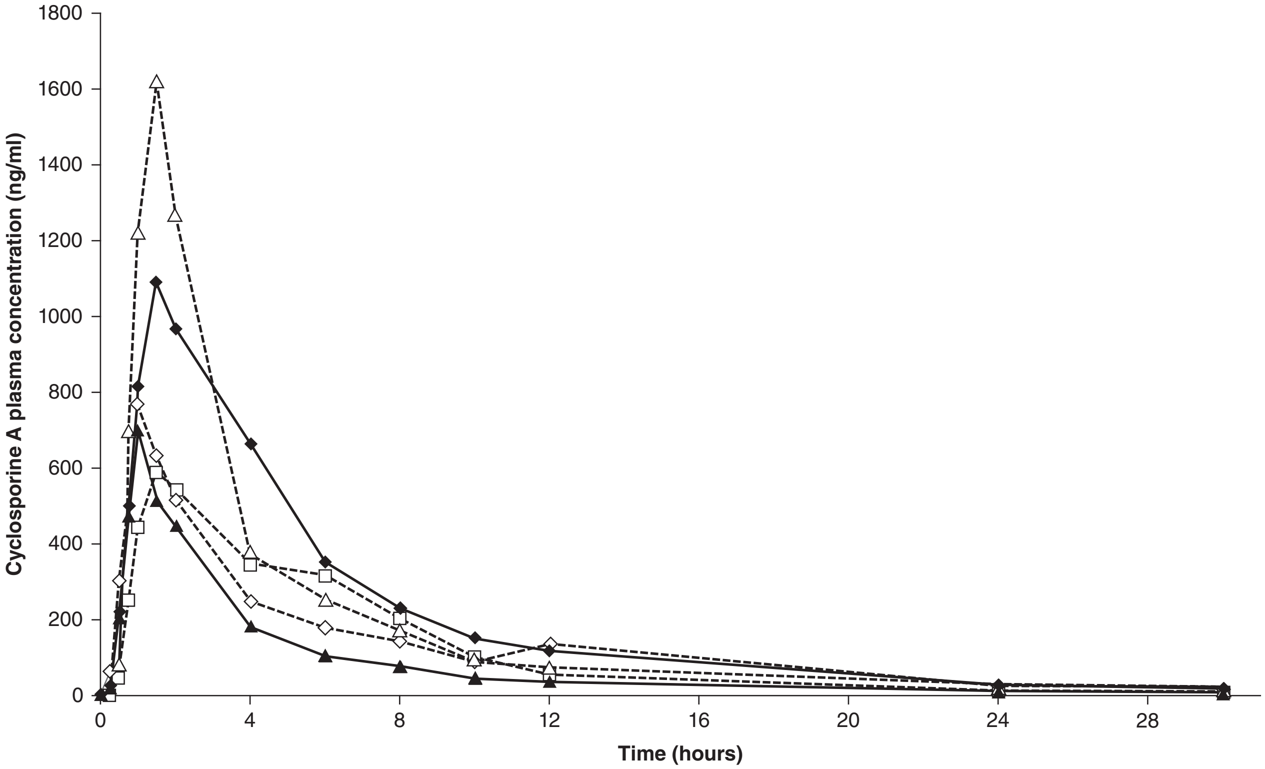


Figure 50.1 Plasma concentration versus time curves of cyclosporine after oral administration (4 mg/kg) to *MDR1* mutant/mutant (open symbols, dashed lines; n = 3) and *MDR1* normal/normal (closed symbols, solid lines; n = 2) Collie dogs.

Oral bioavailability of several P-gp substrates (quinidine, loperamide, nelfinavir, and cyclosporine) were determined in *MDR1* mutant/mutant and *MDR1* normal/normal dogs (Figure 50.1). Contrary to what appears to be the case in other species, there were no differences in oral bioavailability between *MDR1* mutant/mutant dogs and *MDR1* normal/normal dogs for any of the drugs studied (Mise et al., 2004). There are several explanations for the apparent discordant results. First, many P-gp substrates are also CYP3A substrates. Variable CYP3A activity in the study population could have masked differences in P-gp-mediated drug absorption. Second, there may be species differences in P-gp substrate binding and/or efflux kinetics such that oral drug bioavailability is impacted in one species with deficient intestinal P-gp function but not in another. For example, rodents have two genes that encode for P-gp (*abcb1a* and *abcb1b*) while dogs have only one (*ABCB1*). Lastly, at concentrations of drug achieved in the intestine after oral drug administration, P-gp may simply be saturated.

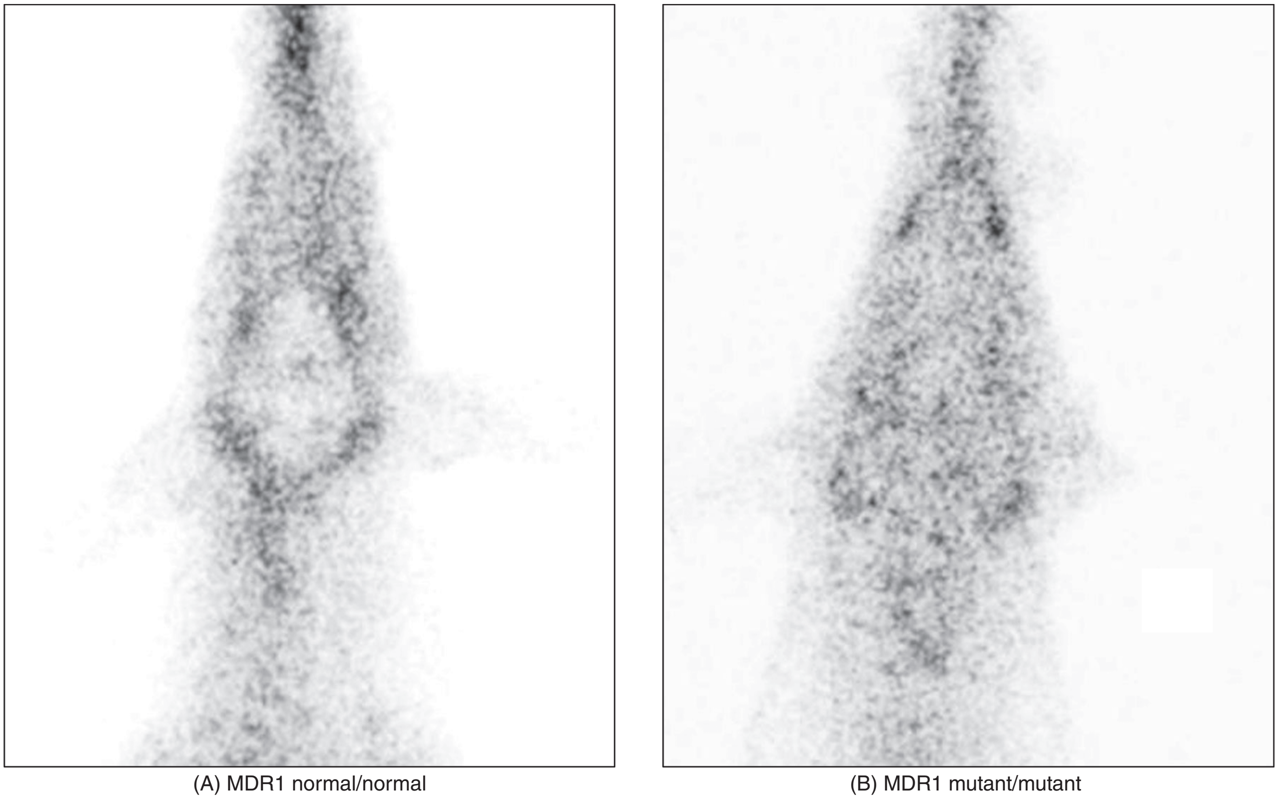
P-glycoprotein is not the only drug transporter that has been shown to affect oral drug absorption in humans and rodents. The breast cancer resistance protein (BCRP), encoded by the *ABCG2* gene, affects oral bioavailability of several drugs in *abcg*2 knockout mice and/or human patients with functional *ABCG2* polymorphisms. Patients with an *ABCG2* variant that alters amino acid 141 have greater oral bioavailability of topotecan than patients with the wild-type allele. Similarly, this variant is associated with greater oral bioavailability of rosuvastatin. Domestic cats, compared to 10 other mammalian species, have defective *ABCG2* function as a result of an *ABCG2* variant that alters amino acid 149 (Court, 2013b). Whether or not this variant affects oral absorption of ABCG2 substrate drugs is not yet known.

Pharmacogenetics of Drug Distribution

Drug distribution, the delivery of drugs from the systemic circulation to tissues, can be dramatically affected by pharmacogenetics. The drug transporter P-gp serves as an important barrier to the distribution of substrate drugs to selected tissues. For example, P-gp is a component of the blood–brain barrier, the blood–testes barrier, and the placenta. Therefore, distribution of P-gp substrate drugs to these tissues is greatly enhanced in dogs with the *MDR1* deletion mutation. Dogs homozygous for the deletion (*MDR1* mutant/mutant) experience adverse neurological effects after a single dose of ivermectin (120 μg/kg). Heterozygous (*MDR1* wild-type/mutant) or homozygous wild-type dogs are not sensitive to ivermectin neurotoxicity at the 120 μg/kg dose, but heterozygote animals may experience neurotoxicity at ivermectin doses greater than 300 μg/kg, particularly if daily doses are administered (i.e., protocols for treatment of demodectic mange). Dogs homozygous for the normal *MDR1* allele (*MDR1* normal/normal), can receive 2000 μg/kg as a single dose without signs of toxicity, and can receive 600 μg/kg/day for months without signs of toxicity. Dogs with the *MDR1* mutation also appear to have increased susceptibility to neurological adverse effects of other avermectins, including milbemycin, selamectin (Revolution® package insert), and moxidectin (Tranquilli et al., 1991). Interestingly, a retrospective study conducted by a national veterinary poison center reported that Collies (the breed with the highest frequency of the *MDR1* mutation) were overrepresented in canine cases of loperamide-induced neurotoxicity (Hugnet et al., 1996). Many Collies displayed signs of neurological toxicity after administration of routinely recommended doses of the antidiarrheal agent loperamide (Imodium®). Loperamide is an opioid that is generally devoid of CNS activity because it is excluded from the brain by P-gp (Ericsson and Johnson, 1990; Wandel et al., 2002). One study demonstrated that dogs with the *MDR1* mutation experienced marked CNS depression after loperamide administration whereas *MDR1* normal/normal dogs experienced no CNS depression (Court et al., 1999).

Figure 50.2 dramatically illustrates P-gp's ability to limit substrate accumulation within the CNS. *MDR1* mutant/mutant and *MDR1* normal/normal dogs received a single intravenous dose of ^{99m}Tc-sestamibi, a radiolabeled P-gp substrate. Nuclear scintigraphy demonstrated minimal uptake of radioactivity in the brain of *MDR1* normal/normal dogs because P-gp actively effluxes the substrate. Sensitive brain tissue is therefore protected from potentially toxic compounds. Conversely, uptake of radioactivity in the brain of *MDR1* mutant/mutant dogs is indistinguishable from surrounding tissues, illustrating the importance of P-gp as a component of the blood–brain barrier (Court et al., 1999). Brain tissue is exposed to much higher concentrations of potentially toxic compounds.

Less information is available regarding P-gp and the blood brain–barrier in cats. The author has recently investigated the feline *ABCB1* gene in cats that experienced ivermectin toxicity after receiving standard doses. A mutation was discovered that would severely impact P-gp function in affected cats (manuscript in preparation). The prevalence of this mutation in the general cat population is estimated to be approximately 5%. Cats affected by this mutation would be expected to


(A) MDR1 normal/normal

(B) MDR1 mutant/mutant

Figure 50.2 Nuclear scintigraphy was used to image the head of two Collies after intravenous injection of the P-gp substrate ^{99m}Tc-sestamibi. The MDR1 normal/normal dog (A) with functional P-gp has prevented accumulation of labeled substrate in the brain, while the MDR1 mutant/mutant dog (B) with defective P-gp function has accumulated the P-gp substrate within brain tissue (Mealey et al., 2008).

have the same phenotype (drug sensitivity) as dogs with the *MDR1* mutation.

Distribution of substrate drugs to the testes and fetus is also limited by P-gp. In human patients, this hampers effective treatment for certain diseases. For example, the testes and brain are considered to be a sanctuary site for the human immunodeficiency virus (HIV)(Choo et al., 2000). Because HIV-1 protease inhibitors are substrates for P-gp, the virus can remain viable in these sanctuary sites, hampering effective therapy. Similarly, therapeutic concentrations of certain chemotherapeutic agents may not be achievable for testicular cancers because of active efflux by P-gp (Katagiri et al., 1993). The effect of placental P-gp on distribution of drugs to the fetus is an area of active research in human medicine (Young et al., 2003). Understanding the role of pregnancy-related hormones in regulating P-gp expression and function is one possible key in developing strategies to deliver drugs to the mother with minimal fetal risk.

Another important drug transporter, briefly mentioned in Section Pharmacogenetics of Oral Drug Absorption, also plays a key role in drug distribution. ABCG2, also known as BCRP, has been studied in several veterinary species including sheep (Polido et al., 2006), cattle (Olsen et al., 2007), and cats (Ramirez et al., 2011). ABCG2 functions as a drug efflux pump in a variety of tissues including the liver, intestine, blood–brain barrier, blood–retina barrier, and mammary gland. ABCG2 dramatically affects concentrations of substrate drugs such as fluoroquinolones in milk (Jonker et al., 2005). A polymorphism in bovine ABCG2 (Tyr581Ser) increases secretion of danoflaxoacin into milk (Otero et al., 2013). After an intramuscular dose of danofloxacin, the AUC and C_{max} of danoflloxacin in milk were twofold lower in cattle with the wild-type *ABCG2* genotype compared to cattle with the *ABCG2* polymorphism. Other fluoroquinolones have also been shown to be actively secreted into milk by ABCG2. Thus, polymorphisms in ABCG2 have implications for drug residues in milk as well as for treatment of mastitis.

Feline ABCG2 has several amino acid changes compared to 10 other mammalian species. One of the amino acid changes is similar to a human variant (ABCG2 421C>A) that results in defective ABCG2 function in people. Feline ABCG2 has decreased ability to transport the “gold-standard” ABCG2 substrate mitoxantrone compared to both human and canine ABCG2 (Ramirez et al., 2011). This has important clinical implications, particularly when one considers that these amino acid changes appear to affect all cats. Consequently, cats would be expected to exhibit enhanced sensitivity to ABCG2 substrate drugs compared to other species.

This is obviously the case with certain fluoroquinolones. Domestic cats experience an unusual, and apparently species-specific, adverse drug reaction associated with fluoroquinolones – acute retinal degeneration and blindness (Wiebe and Hamilton, 2002). Fluoroquinolone-induced retinal toxicity was first documented with enrofloxacin, which is structurally similar to the human-approved fluoroquinolone ciprofloxacin. In a prospective study, 100% of cats that received enrofloxacin (10 × label dose) developed retinal degeneration as early as 3 days after treatment while 0% of control cats developed retinal lesions (Ford et al., 2007). Orbifloxacin, another fluoroquinolone approved for use in cats and dogs, also causes retinal damage in cats (FDA, 2006). Retinal degeneration and blindness can result from extensive phototoxic damage to the retina. When exposed to light, fluoroquinolones generate reactive oxygen species (ROS) that attack cellular lipid membranes causing tissue damage (Agrawal et al., 2007). Under normal conditions, the retina is protected from photoreactive compounds by the blood–retina barrier, including transporters such as ABCG2 that function to prevent distribution of xenobiotics to the retina (Asashima et al., 2006). As substrates for ABCG2, fluoroquinolone distribution to the retina is normally restricted by ABCG2. Dysfunction of ABCG2 in cats disrupts the blood–retina barrier, allowing accumulation of photoreactive fluoroquinolones. Exposure of the retina to light then generates ROS that damage tissues resulting in retinal degeneration and blindness.

The impact of feline ABCG2 dysfunction on other ABCG2-substrate drugs has not yet been investigated. Whether or not ABCG2 polymorphisms exist in other common veterinary species such as dogs or horses has not yet been documented.

Pharmacogenetics of Drug Metabolism

Presently, the greatest body of knowledge with regard to pharmacogenetics in human patients involves genetic variation in drug metabolizing enzymes. Pharmacogenetic variation can affect both Phase I and Phase II metabolic enzyme activity. A mutation in the gene encoding pseudocholinesterase serves as an example of how pharmacogenetic variation can result in dramatic differences in drug response between patients. Patients with a normal pseudocholinesterase genotype metabolize succinylcholine and recover from neuromuscular blockade rapidly; those with the mutant genotype undergo sustained neuromuscular blockade that can result in prolonged apnea and the necessity for mechanical ventilation (Wing, 1974). A number of polymorphisms have been described in human cytochrome P450 (CYP) enzymes, many of these resulting in profound variations in clinical response. For example CYP2D6 is a highly variable P450 pathway in humans with individuals ranging from undetectable activity (found in 6–10% of Caucasians) to “ultrarapid” activity (found in 3–10% of Europeans and 30% of one black population)(Cascorbi, 2003). The ultrarapid phenotype is due to a highly unusual gene duplication. Drugs that are substrates for CY2D6 in people include β-receptor antagonists (propranolol, timolol, metoprolol), antiarrhythmics (quinidine, flecainide), antidepressants (amitriptyline, clomoipramine, fluoxetine, imipramine), neuroleptics, and certain opioid derivatives. Depending on the patient's CYP2D6 genotype, the "typical" dose of a substrate drug may need to be decreased (poor metabolizers require 1/10 of the standard dose of nortriptyline to avoid toxicity) or increased (ultrarapid metabolizers require five times the standard dose to achieve therapeutic concentrations).

Canine CYP1A2

Compared to people, relatively few polymorphisms in drug metabolizing enzymes have been described in veterinary species. However, several polymorphisms have now been described in canine CYPs. The most well-characterized canine CYP polymorphism is a mutation (c1117C>T) in the coding region of *CYP1A2* that generates a premature stop codon. Homozygously affected dogs lack hepatic CYP1A2 protein expression and enzymatic function (Tenmizu et al., 2004; Mise et al., 2004).

Screening for the polymorphism in populations of beagles in Japanese research institutes identified approximately 11–17% of the dogs as homozygous for the mutant genotype and consequently lacking functional CYP1A2. Plasma concentrations of investigational drugs were up to 17 times greater in dogs with deficient CYP1A2 (Tenmizu et al., 2004; Mise et al., 2004). Plasma drug concentrations in dogs that were heterozygous for *CYP1A2* c1117C>T were not substantially different from concentrations in wild-type dogs. Whether or not this polymorphism impacts the pharmacokinetics, efficacy, and safety of clinically used drugs is not known and would depend on: (i) the presence of *CYP1A2* 1117C>T in the general canine patient population; and (ii) the degree to which a drug depended on CYP1A2 for clearance. A review article summarized the results of several studies investigating the allele frequency of *CYP1A2* 1117C>T (Court, 2013a). Breeds with the highest allele frequency include Irish Wolfhounds, Beagles, and Berger Blanc Suisse. Importantly, Beagles from Japan had a much higher allele frequency than Beagles from either Europe or the United States. Many of the remaining breeds that were found to harbor the mutation, albeit at allele frequencies <10% were the same herding breeds that are known to harbor *ABCB1-1*Δ, the mutation that results in P-gp deficiency.

Canine CYP2B11

A phenotype involving anesthetic sensitivity has been described for Greyhounds and other sighthound breeds. Specifically, dogs of this breed have been reported to have prolonged recovery from the injectable anesthetic agents including thiopental, thiamylal, and propofol. Many years ago this was attributed to decreased drug redistribution from the central compartment due to the lean nature of the affected breeds. Subsequently, researchers have identified decreased drug clearance in these dogs that was preventable by pretreating the dogs with the CYP inducer phenobarbital, implicating CYP involvement. Studies using dog liver microsomes identified CYP2B11 as the primary enzyme responsible for CYP-dependent clearance of propofol (Court et al., 1999; Hay Kraus et al., 2000). A specific molecular mechanism for this breed-dependent difference in drug disposition has not yet been reported.

Miscellaneous Canine CYP Polymorphisms

A canine CYP2C isoform, CYP2C41, was identified in a small percentage of beagles and mixed breed dogs suggesting that the majority of dogs have a partial or complete deletion of this enzyme. The CYP2C21 isoform was expressed in all dogs examined and because this isoform metabolizes many of the same drugs as CYP2C41, the impact of the canine CYP2C41 deletion on drug disposition may be minimal (Court, 2013a). Amino acid variants, likely the result of SNPs, involving canine CYP2D15, CYP2E1, and CYP3A12, have been reported. Further investigation is required to determine the allele frequency and the potential impact these variants might have on the disposition of various drugs in the clinical setting.

CYP Polymorphisms in Other Species

A feline hepatic CYP2E polymorphism has been identified, but the clinical relevance of this polymorphism has not been described (Tanaka et al., 2005). Similarly, polymorphisms have been described in several drug-metabolizing enzymes in cattle (Theilmann et al., 1989), but these single nucleotide polymorphisms are used as molecular markers available in cattle for linkage analysis, testing of parentage, and distinction of breeds, rather than for predicting response to drug therapy.

Phase II Metabolic Enzymes

With respect to Phase II metabolic enzymes, a panspecies defect in UDP-glucuronyl transferase (UGT) enzymes exists in cats and these defects dramatically affect drug disposition in that species. Because these defects represent a genetic variation between species rather than within a species, these are not considered true pharmagenetic defects. Cats lack several, but not all, UGTs. The UGTs lacking in cats are responsible for metabolizing simple planar phenolic compounds (e.g., acetaminophen and other phenolic compounds) (Court, 2013b). This defect partially explains the pharmacological idiosyncrasies of cats relative to most common veterinary species.

Another panspecies Phase II metabolic defect occurs in dogs. *N*-acetyltransferase is the enzyme responsible for metabolizing sulfonamides, procainamide, hydralazine, and other drugs. Both *N*-acetyltransferase genes (*NAT1* and *NAT2*) are absent in dogs, increasing the risk for hypersensitivity reactions and adverse effects from these drugs relative to other species (Trepanier et al., 1997). *NAT2* deficiency contributes to low clearance of some sulfonamides. Cats lack *NAT2* but express *NAT1* (Court, 2013b).

A true pharmacogenetic variation exists for the thiopurine methyltransferase (TPMT) enzyme. TPMT is a Phase II enzyme that is responsible for metabolizing azathioprine and its active metabolites to their inactive forms. A ninefold range in TMPT activity exists in dogs, and enzyme activity level appears to be related to breed. Giant Schnauzers had lower TPMT activity while Alaskan Malamutes had high TMT activity (Kidd et al., 2004). Decreased TPMT activity has been documented to be associated with increased susceptibility to azathioprine-induced bone marrow suppression.

Pharmacogenetics of Drug Excretion

Drugs are eliminated from the body either unchanged or as metabolites. Renal and biliary excretion are the most important pathways of drug elimination, but excretion may occur by other routes as well.

P-glycoprotein Mediated Biliary Excretion

As noted previously, P-gp is expressed on renal tubular cells and biliary canalicular cells, suggesting that it may play a role in drug excretion. The *ABCB1-1*Δ mutation dramatically impacts biliary drug excretion. Studies using the radiolabeled P-gp substrate $^{99\mathrm{m}}$Tc-sestamibi in *MDR1* mutant/mutant and *MDR1* normal/normal dogs illustrate the importance of P-gp-mediated biliary excretion of compounds that are P-gp substrates. *MDR1* mutant/mutant dogs have significantly decreased biliary excretion of $^{99\mathrm{m}}$Tc-sestamibi compared to *MDR1* normal/normal dogs (Coelho et al., 2009). *MDR1* mutant/normal dogs have an intermediate phenotype. *MDR1* mutant/mutant dogs appear to have no biliary excretion of $^{99\mathrm{m}}$Tc-sestamibi (Figure 50.3). Thus, blood and tissue concentrations of P-gp substrates are likely to persist longer in *MDR1* mutant/mutant dogs compared to *MDR1* normal/normal dogs, increasing the risk for adverse drug reactions. Because clearance of both vincristine and doxorubicin depend on P-gp mediated biliary excretion, these drugs serve as examples of P-gp substrate drugs that carry a higher risk for toxicity in *MDR1* mutant/mutant dogs compared to *MDR1* normal/normal dogs.

A prospective study examined vincristine-associated hematological toxicity in dogs with lymphoma. Dogs that were either heterozygous or homozygous for *ABCB1-1*Δ were significantly more likely to develop neutropenia and thrombocytopenia than wild-type dogs (Mealey et al., 2008). The author is aware of two *MDR1* mutant/mutant dogs that died from complications of sepsis after receiving doxorubicin (30 mg/m^2). Both dogs experienced severe GI toxicity and severe neutropenia within 10 days of receiving doxorubicin. Initiating a prospective study to specifically define the effects of doxorubicin in dogs with the *MDR1* mutation was not pursued for obvious ethical reasons. Other reports of severe doxorubicin, vinblastine, and/or vincristine sensitivity in Collies and other herding breeds have surfaced on internet veterinary discussion groups. Thus, genotyping herding breed dogs and mixed breed dogs prior to treatment with P-gp

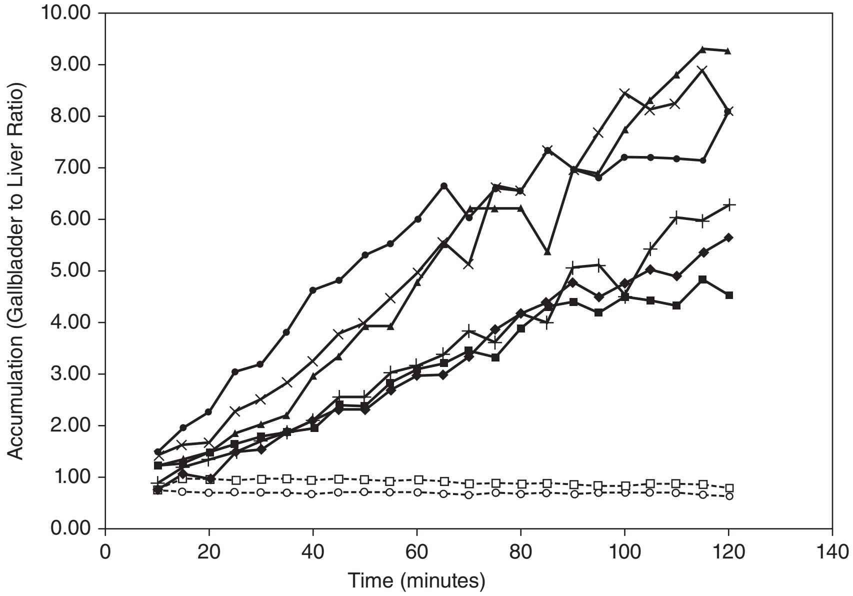


Figure 50.3 Accumulation of the P-gp substrate $^{99\mathrm{m}}$Tc-sestamibi over time in the gallbladder of six *MDR1* mutant/mutant (solid lines) and two *MDR1* normal/normal dogs (dotted lines) after intravenous injection of $^{99\mathrm{m}}$Tc-sestamibi. Note that *MDR1* mutant/mutant dogs are essentially unable to excrete P-gp substrates.

substrate chemotherapeutic drugs has become the standard of care in veterinary oncology. Based on the individual patient's *MDR1* genotype, appropriate dose reductions can be made so that the patient experiences the antitumor effect of these drugs but avoids severe toxicity.

Solute Carrier-Mediated Renal Excretion

The solute carrier (SLC) superfamily of transporters consists of both uptake transporters (transport substrates into cells) and efflux transporters (transport substrates out of cells). In humans and rodents, members of the SLC superfamily transport a variety of endogenous and exogenous compounds (Roth et al., 2012). There is some overlap of substrates among members of the SLC superfamily in humans and rodents. For example, fluvastatin (cholesterol-lowering drug) is a substrate for at least three different members of the human SLC superfamily. Less is known about substrate specificity of veterinary SLC transporters. In humans, rodents, and presumably other veterinary species, SLC transporters are expressed along the body's functional "barrier" tissues including intestine, brain capillary endothelial cells, placenta, liver, and kidney as well as other tissues. These strategic locations allow SLC transporters to influence not only renal drug excretion, but drug absorption, distribution, and metabolism as well. Members of the SLC superfamily include organic cation transporters (OCTs), multidrug and toxin extrusion transporters (MATEs), organic anion transporters (OATs), and organic anion transporting polypeptides (OATPs). Genetic variation in OCTs has been documented in human patients (Franke et al., 2010). Plasma concentrations of the antiemetic ondansetron are higher and clinical effects greater in human patients with *OCT1* mutations. The author is not aware of genetic variants in the SLC superfamily in veterinary species that have been associated with changes in drug disposition.

Pharmacogenetics of Drug Receptors

A more recently explored area of pharmacogenetics involves identifying polymorphisms in genes encoding drug receptors and effector proteins. In human patients, polymorphisms have been described in angiotensin converting enzyme (ACE), β-adrenergic receptors, the dopamine receptor, the estrogen receptor, and others (Tribut et al., 2002). One polymorphism in the *ACE* gene in patients with heart failure is associated with an increased risk of death or the need for transplantation (Joseph et al., 2014). Polymorphisms of the human β_1-adrenergic receptor (ADRB1) have been shown to have either enhancing or suppressing effects on adenylyl cyclase activity, depending on the particular polymorphism. Because effects of individual polymorphisms on clinical outcomes have not been conclusive, pharmacogenetic testing for ADRB1 polymorphisms has not been adopted into clinical cardiology practice.

Two independent in-frame deletion mutations have been described in canine ADRB1 (Maran et al., 2013). Preliminary results suggest that dogs that harbor both of these deletions are less responsive to the effects of the β-adrenergic antagonist atenolol. Polymorphisms of feline ADRB1 have also been described but the functional significance has not yet been determined (Maran et al., 2012).

A polymorphism in the canine phosphodiesterase 5A (*PDE5A*) gene has been described (Stern et al., 2014). Vasodilators such as nitric oxide and sildenafil interact with PDE5A. Plasma concentrations of cGMP were significantly lower in dogs with the PDE5A polymorphism compared to wild-type dogs. Whether or not affected dogs respond differently to nitric oxide or sildenafil has yet to be determined.

Pharmacogenetics and Idiosyncratic Reactions

Pharmacogenetic differences in metabolic pathways can affect not only Type A adverse drug reactions (predictable; generally correlating with plasma drug concentration), but can also affect type B adverse drug reactions (idiosyncratic). Idiosyncratic reactions are sometimes called hypersensitivity reactions but they do not always involve an adaptive immune response. One of the most common drug classes involved in idiosyncratic adverse reactions in both dogs and people are the sulfonamides and can be characterized by fever, arthropathy, blood dyscrasias (neutropenia, thrombocytopenia, or hemolytic anemia), hepatopathy consisting of cholestasis or necrosis, skin eruptions, uveitis, or keratoconjunctivitis sicca (Trepanier, 2004). In people, slow acetylation by NAT2 has been shown to be a risk factor for sulfonamide hypersensitivity reactions. It has been proposed that alternative metabolic pathway in these individuals generate reactive metabolites. Covalent binding of these reactive metabolites to cell macromolecules results in cytotoxicity and immune response to neoantigens. While a specific genetic variant or variants have not been identified in dogs experiencing sulfonamide hypersensitivity, a study has ruled out the sulfonamide detoxification genes *NAT2*, *CYB5A*, and *CYP5R3* (Sacco et al., 2012).

Pharmacogenetics and Protein Binding

Several plasma proteins can bind xenobiotics but albumin is usually considered to be among the most important with regard to drugs used in veterinary medicine. Because only the unbound fraction of drug is pharmacologically active, changes in protein binding can affect efficacy and toxicity of highly protein-bound drugs. The pharmacokinetics of a drug can also be altered with changes in the degree of protein binding. Since only the unbound fraction is generally available for metabolism and/or excretion, clearance of a highly protein-bound drug may be increased in situations that increase the unbound fraction of that drug. Situations that can alter protein binding of a drug for an individual patient include: drug interactions (coadministration of two highly protein-bound drugs); and changes in plasma albumin concentration (disease states causing hypoalbuminemia or rapid increases in plasma albumin resulting from administration of albumin solutions). Pharmacogenetics may account for differences in protein binding between patients.

For example, two linked SNPs (G1075T and A1422T) in the canine albumin gene were shown to correlate with protein binding and clearance of an investigative drug (Takashi et al., 2009). The SNPs results in an amino acid changes (Ala335Ser and Glu450Asp) that appear to alter binding of the investigative compound, an integrin antagonist. The prevalence of the mutant allele in a research beagle colony was 40% (n = 47). The drug is >99% protein bound in all dogs with the mean unbound fraction in homozygous wild-type beagles (0.014%), significantly lower than in beagles homozygous for the mutant allele (0.053%). Clearance of the drug was significantly greater in beagles homozygous for the mutant allele (3.66 ml/min/kg) than in beagles homozygous for the wild-type allele (1.97 ml/min/kg). For both measurements heterozygous beagles were intermediate. Further investigation is necessary to determine: (i) if this mutation is present in dogs (beagles and other breeds) outside this colony; and (ii) if other highly protein-bound drugs are affected by this allele.

Pharmacogenetics in Clinical Practice

Clinical use of pharmacogenetic testing for human patients is becoming standard practice for some drugs. In fact, pharmacogenetic testing is now required by the FDA for some drugs, and for others, the label includes information regarding genetic variations that impact the drug's use (Pacanowski et al., 2014).There are over two dozen pharmacogenetic tests available for human patients. Some of these pharmacogenetic tests may improve selection of patients for which a particular drug will be effective while others may predict which patients are more likely to experience an adverse reaction. Pharmacogenetic testing for oncological and cardiovascular drugs seems to be the most readily available. For example, pharmacogenetic testing is available for the anticancer drugs 5-fluorouracil, irinotecan, herceptin, and tyrosine kinase inhibitors and FLT3 inhibitors. Pharmacogenetic testing for the "blood thinners" clopidogrel and warfarin is also readily available. Pharmacogenetic testing for individual drug metabolizing enzymes (CYP2D6, CYP2C19, CYP3A4 and 5, NAT2, and others) is also available. Thus, it is possible for a physician to obtain a complete CYP profile on a patient. However, specific dosing recommendations are not currently available for most drugs despite knowing a patient's CYP profile, so the clinical applications of such testing may be limited.

In veterinary medicine, a commercial veterinary pharmacogenetics laboratory (Veterinary Clinical Pharmacology Laboratory, Washington State University, Pullman, WA, 99164; www.vetmed.wsu.edu/vcpl) is performing canine *MDR1* (*ABCB1-1*Δ) genotyping for veterinarians, dog breeders, and owners. Pharmacogenetic testing is considered standard-of-care for some canine patients. Dogs for which vincristine, vinblastine,

Table 50.1 Selected P-gp substrates. Source: Data from Schwab et al., 2003; Fromm, 2002; Sakaeda et al., 2002; Sakaeda et al., 2003; Marzolini et al., 2004.

Anticancer agents
Doxorubicin
Docetaxel[a]
Vincristine[a]
Vinblastine[a]
Etoposide[a]
Mitoxantrone
Actinomycin D
Steroid hormones
Aldosterone
Cortisol[a]
Dexamethasone[a]
Methylprednisolone
Antimicrobial agents
Erythromycin[a]
Ketoconazole
Itraconazole[a]
Tetracycline
Doxycycline
Levofloxacin
Sparfloxacin
Opioids
Loperamide
Morphine
Cardiac drugs
Digoxin
Diltiazem[a]
Verapamil[a]
Talinolol
Immunosuppressants
Cyclosporine[a]
Tacrolimus[a]
Miscellaneous
Ivermectin
Amitriptyline
Terfenadine[a]
Ondansetron
Domperidon
Phenothiazines
Vecuronium

[a] Substrate of CYP 3A.

doxorubicin, loperamide, or ivermectin (extralabel dose for treating mange) are being considered should be tested for the *MDR1* mutation prior to dosing. See Table 50.1 for a partial list of drugs that are substrates for P-gp. Because the *MDR1* mutation in dogs has a very high allelic frequency (55% in Collies; 42% in Longhaired Whippets; and roughly 20% in Australian Shepherds), and because the polymorphism is highly predictive for serious adverse drug events including death, pharmacogenetic testing in dogs has been readily accepted by veterinarians.

Future Directions

The *MDR1* test for dogs demonstrates that the relatively new field of pharmacogenetics has already made an impact on drug safety and efficacy for veterinary patients. As is the case with any novel medical procedure, it will take time for pharmacogenetics to be considered a standard tool for making treatment decisions. Because the FDA already screens drugs for safety and efficacy in large patient populations, pharmacogenetics, by default, benefits a minority of the population. However, by using pharmacogenomics as a tool for making therapeutic decisions, veterinarians will be able to provide better overall treatment by optimizing therapy for each patient (choosing the right dose of the right drug the first time — rather than by trial and error). The ultimate goal of pharmacogenetics is to enable patients to respond to treatment faster, at less expense, and with a much lower risk for adverse effects.

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51

Considerations for Treating Minor Food-Producing Animals with Veterinary Pharmaceuticals

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The Use of Veterinary Pharmaceuticals in Minor Food-Producing Animals: Special Considerations and Challenges

Antibiotics, antiparasitics, nonsteroidal antiinflammatory drugs, and exogenous hormones are among the essential components in the armamentarium of a food animal veterinarian to combat disease, alleviate animal pain, and minimize economic losses. In the United States, many of these veterinary products are US Food and Drug Administration (FDA) approved for use in a large number of major food-producing animals but not for the minor food-producing animals. According to the FDA, the major food-producing animals are cattle (meat and milk), swine, chickens (meat and eggs), and turkeys. The minor food-producing animals are those that do not fall into the major food-producing animal category with the most popular being goats (both dairy and meat), sheep, deer, commercially raised game birds, and food fish and shellfish. This category also includes less obvious food-producing species such as rabbits, ratites, and honey bees. In the USA, horses are a major animal species; however, they are considered companion animals and are not customarily used for human food as in some other countries. In Europe, the European Medicines Agency has defined the major food-producing animals to be cattle (meat and milk), sheep (meat); pigs; chickens (meat and eggs), and salmonids. Minor food-producing animals include minor ruminants (meat and milk), deer (including reindeer), other avian species (meat and eggs), other fish species, and other mammalian species (horse and rabbit). In other countries, the minor food-producing animal category has the potential to be considerably larger because a greater variety of species are consumed such as small rodents, canids, invertebrates, and various avian species.

In recent years, there has been an increased interest in consuming more meat and other products from minor food-producing animals, especially aquaculture species. As this interest grows, so will the expectations to provide safe food items for human consumption. Maintaining the overall health of minor food-producing animals is similar to managing the health of major food-producing animals, and typically requires some use of veterinary pharmaceuticals. However, in contrast to the major food-producing animals, few drugs have FDA approval for use in minor food-producing animals.

One of the major challenges for getting veterinary products approved for use in minor food-producing animals is the reduced economic return due to small market size. Additionally, some of the minor food-producing animals are less tractable (e.g., cervids and bison), and there is wide variation in breeds/species with respect to size and pharmacokinetics. In general, there is a large number of species and incredible diversity within this category. For aquatic species, such as finfish, mollusks, and crustaceans, not only are there species differences, but environmental considerations (salinity, pH, temperature), physiological differences (heterothermy), and management practices that also impact metabolism and excretion of drugs.

This chapter addresses the unique issues associated with the regulatory requirements for getting a drug approved for use in a minor food-producing animal, the availability of veterinary pharmaceuticals for use in minor food-producing animals, and highlights legislation that has helped address the veterinarian's need for medications to treat these animals. In addition, government programs and scientific approaches used to meet the therapeutic needs for treating minor food-producing animals are discussed.

The Approval Process for Veterinary Pharmaceuticals for Use in Minor Food-Producing Animals

There are many veterinary pharmaceuticals marketed today that may be administered to minor food-producing animals. The ideal scenario for a veterinarian wanting to

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treat a minor food-producing animal is having an FDA-approved drug labeled for the intended use in question. One example would be an anthelmintic approved for treating specific gastrointestinal parasites in sheep. This product will have been demonstrated to FDA's satisfaction to be safe for sheep, safe for the person administering the drug to the sheep, safe for anyone who later consumes meat from the treated sheep, and safe for the environment, both during the drug's use and its disposal. It will also have been shown to be effective for the labeled use and that it is consistently manufactured to standards of strength and purity. Such a product is labeled with intended uses, doses, and withdrawal times that are appropriate for use in sheep.

In contrast to the major food-producing animals, there are not many FDA-approved drugs labeled for use in minor food-producing animals. This is mainly because the development of new drugs for use in animals is a very expensive and time-consuming process, especially for drugs to be used in animals intended for human consumption, and the minor food-producing animal markets are too small to readily recover the pharmaceutical sponsor's investment. FDA-approved drugs for minor food-producing animals (as of July 2017) are summarized in Tables 51.1–51.5. There is also a searchable database of approved new animal drugs available on the FDA website. Please note that even if approved, a particular product may not be commercially available. This can be for a variety of reasons including closing of manufacturing facilities, or the discontinuation of less lucrative product lines when two companies merge.

For a drug to be approved for use in a minor food-producing animal, the sponsor must demonstrate to the satisfaction of the scientific reviewers at FDA's Center for Veterinary Medicine (CVM) that it is safe and effective for its intended use. CVM is the component of FDA responsible for the regulation of veterinary drugs, including the evaluation of such drugs prior to approval.

The information that a sponsor needs to present to support a New Animal Drug Application (NADA) includes the following as outlined in regulations published in the Code of Federal Regulations (21 CFR 514):

1) effectiveness for the proposed use;
2) safety to the target animal species (the animal species for which the drug is to be used);
3) human food safety (safety to consumers of meat, milk, or eggs derived from treated animals);
4) environmental safety;
5) manufacturing methods and controls;
6) all other information;
7) freedom of information (FOI) summary; and
8) product labeling.

These components are discussed in detail in Chapter 55 of this book. The FDA also provides a variety of guidance documents to assist sponsors in understanding the various requirements. Of particular note for sponsors seeking drug approval for a minor species use is *Guidance #61 FDA Approval of New Animal Drugs for Minor Uses and for Minor Species*. This document is being revised at the time of writing this chapter and will be published under the new title, *Special Considerations, Incentives, and Programs to Support the Approval of New Animal Drugs for Minor Uses and for Minor Species*. Guidance documents are available on the FDA website.

Compared to the drug approval process for major food-producing animals, the drug approval process is not significantly easier for products intended for use in a minor food-producing species. The same high standards must be met. However, there are a few advantages afforded to products intended for use in a minor food-producing animal. These include the possibility of a waiver from user fees, the ability to apply conclusions reached from the radiolabeled residue study in the major food-producing animal to the human food safety evaluation for the minor food-producing animal, the possibility of a categorical exclusion from the need to provide an environmental assessment, and the possibility that fewer sites will be needed for effectiveness clinical trials.

Legislation and Policies Supporting the Availability of Veterinary Pharmaceuticals in Minor Food-Producing Animals

Animal Medicinal Drug Use Clarification Act

The US Congress recognized the drug availability problem for both major and minor animal species when it passed the Animal Medicinal Drug Use Clarification Act (AMDUCA) in 1994 (Public Law No. 103–396; Monti, 2000). This law addressed the fact that FDA generally approves veterinary drugs for narrow intended uses. For example, a broad-spectrum antibiotic that would be safe and effective for the treatment of a wide variety of bacterial infections may only be *approved* for use in cattle for the treatment of respiratory disease caused by specific bacteria. From a practical standpoint, the pharmaceutical sponsor cannot demonstrate safety and effectiveness for every possible intended use of the product. This means that veterinarians frequently administer the drug for indications that are not on the label. In other words, they use the drug for different diseases, at different doses, by different routes of administration, and in different animal species than what is indicated on the label.

AMDUCA legalized this practice for veterinarians within a set of limitations described in the Code of Federal Regulations (21 CFR 530). In general, veterinarians, within the context of a valid veterinarian–client–patient

Table 51.1 Veterinary pharmaceuticals approved by the Food and Drug Administration as of July 2017 for use in small ruminants that are considered minor food-producing animals in the United States of America

Drug	Formulation	Species	Indication[a]
Albendazole	Liquid suspension	Sheep Goats	Internal parasites Liver flukes
Ceftiofur sodium	Injection	Goats Sheep	Respiratory infection
Chlortetracycline	Premix (for feed)	Sheep	Vibrionic abortion Claim withdrawn
Decoquinate	Premix (for feed)	Goats Sheep	Coccidiosis
Fenbendazole	Liquid suspension	Goats	Internal parasites
Fenbendazole	Premix (for feed)	Zoo/wild goats Bighorn sheep	Internal parasites
Flurogestone acetate	Vaginal sponge	Sheep	Estrus synchronization
Follicle stimulating hormone	Injectable—IV, IM, SQ	Sheep	FSH deficiency
Ivermectin	Drench/liquid	Sheep	Internal parasites
Lasalocid	Premix (for feed)	Sheep	Coccidiosis
Levamisole hydrochloride	Drench Bolus Powder	Sheep	Internal parasites
Monensin sodium	Premix (for feed)	Goats	Coccidiosis
Morantel tartrate	Premix (for feed)	Goats	Internal parasites
Methoxyflurane	Inhalation	Sheep	Anesthetic
Moxidectin	Drench	Sheep	Internal parasites
Neomycin sulfate	Powder Liquid	Goats Sheep	Colibacillosis
Neomycin sulfate	Premix (for feed)	Sheep	Colibacillosis
Neostigmine methylsulfate	Injectable—SQ	Sheep	Rumen atony Curare antagonist
Oxytetracycline	Premix (for feed) Powder	Sheep	Respiratory infection Colibacillosis Claim withdrawn
Oxytetracycline hydrochloride—Polymyxin B Sulfate	Ointment	Sheep	Superficial ocular infections
Oxytocin	Injection—IM, IV, SQ	Sheep	Uterine contractions Milk letdown
Penicillin G procaine	Injectable—IM	Sheep	Respiratory infection
Pituitary luteinizing hormone	Injectable—IV, SQ	Sheep	Pituitary hypofunction
Progesterone	Intravaginal device (CIDR)	Sheep	Out-of-season breeding
Proparacaine hydrochloride	Liquid	Sheep	Ophthalmic anesthetic
Sodium chloride gelatin	Oral liquid	Sheep	Shock/hypovolemia
Sodium selenite/ Vitamin E	Injectable—IM, SQ	Sheep	Selenium deficiency
Tetracycline	Injectable—IM	Sheep	Bacterial infection
Thiabendazole	Drench Premix (for feed) Pellet Liquid	Goats Sheep	Internal parasites
Thialbarbitone sodium	Powder—injectable IV	Sheep	General anesthetic
Tilmicosin phosphate	Injection	Sheep	Respiratory infection
Zeranol	Implant—SQ	Sheep	Weight gain/feed efficiency

[a] Listed indications are broad. For specific indications, the drug label or Code of Federal Regulations should be consulted.

relationship, may use approved dosage form products (but not medicated feeds) outside their labeling. This is allowed only when there is no approved drug for the intended use; other approved drugs are not effective for such use, when the dose is not effective for that use, or in cases where an approved formulation is not appropriate. When using a drug outside of its labeling for a food-producing animal, the veterinarian is responsible for using the drug in a manner that will not result in a violative residue in any food product derived from that animal. The veterinarian must establish a withdrawal period based on some scientific information, although

Table 51.2 Veterinary pharmaceuticals approved by the Food and Drug Administration as of July, 2017 for use in cervids/other ruminants that are considered minor food-producing animals in the United States of America

Drug	Formulation	Species	Indication[a]
Ivermectin	Injection	Bison (American) Reindeer	Hypodermosis Warbles
Diprenorphine hydrochloride Etorphine hydrochloride	Injectable – IM, IV	Wildlife	Immobilization
Xylazine hydrochloride	Injectable—IM, IV	Deer Elk	Sedation
Yohimbine hydrochloride	Injectable—IV	Deer Elk	Xylazine antagonist

[a] Listed indications are broad. For specific indications, the drug label or Code of Federal Regulations should be consulted.

there are few resources for establishing such times. The withdrawal period may be based on data in published literature, extrapolated from the pharmacokinetic profile of the drug, or determined through consultation with organizations such as the Food Animal Residue Avoidance and Depletion Program (FARAD). There are also certain drugs not eligible for extralabel use in food-producing animals. These are listed in the CFR at 530.41. This list includes a fairly new ban on the extralabel use of most cephalosporins. This ban applies only to the major species – cows, pigs, chickens, and turkeys.

Compliance Policy Guide 615.115

Because the extralabel use of medicated feeds is prohibited by law, an undue hardship was placed on minor food-producing animals that could not be practically medicated in any other way. An example would be commercially raised game birds, such as pheasants, that are raised in very large outdoor pens. If these birds become ill, they cannot be dosed individually and they will drink water from puddles rather than medicated water. Therefore, they are most often treated via the feed. Aquaculture species are another group of animals best treated with medicated feed. Given that there are few drugs approved for these species (and some of these not marketed) a means of relief was provided through a compliance policy guide (CPG). This CPG is FDA's direction to field investigators when and how to exercise enforcement discretion in these cases. The CPG does not make extralabel use of medicated feed legal and a CPG can be rescinded at any time. It simply means that if veterinarians, feed mills, and producers follow the parameters laid

Table 51.3 Veterinary pharmaceuticals approved by the Food and Drug Administration as of July, 2017 for use in avian species that are considered minor food-producing animals in the United States of America

Drug	Formulation	Species	Indication[a]
Amprolium	Premix (for feed)	Pheasants	Coccidiosis
Bacitracin methylene disalicylate	Premix (for feed)	Pheasants Quail	Weight gain/feed efficiency
Bacitracin methylene disalicylate	Water-soluble powder	Quail	Ulcerative enteritis
Bacitracin zinc	Premix (for feed)	Pheasants Quail	Weight gain/feed efficiency
Bacitracin zinc	Water-soluble powder	Quail	Ulcerative enteritis
Carnidazole	Tablets	Pigeons	Trichomoniasis
Chlortetracycline	Premix (for feed)	Ducks	Fowl cholera
Iodinated casein	Premix (for feed)	Ducks	Weight gain/feathering
Lasalocid	Premix (for feed)	Partridge (Chukar)	Coccidiosis
Monensin sodium	Premix (for feed)	Quail (Bobwhite)	Coccidiosis
Novobiocin	Premix (for feed)	Ducks	Bacterial infection
Salinomycin sodium	Premix (for feed) Powder	Quail	Coccidiosis
Sulfadimethoxine ormetoprim	Premix (for feed)	Ducks Partridge (Chukar)	Bacterial infection Coccidiosis
Thiabendazole	Premix (for feed)	Pheasants	Gapeworm

[a] Listed indications are broad. For specific indications, the drug label or Code of Federal Regulations should be consulted.

Table 51.4 Veterinary pharmaceuticals approved by the Food and Drug Administration as of May, 2014 for use in aquaculture species in the United States of America

Drug	Formulation	Species	Indication[a]
Florfenicol	Premix (for feed)	Catfish	Enteric septicemia of catfish
Florfenicol (now fully approved)	Premix (for feed)	Catfish	Columnaris disease
Florfenicol	Premix (for feed)	Freshwater-reared Salmonids	Coldwater disease Furunculosis
Florfenicol	Premix (for feed)	Freshwater-reared finfish	Streptococcal septicemia Columnaris disease
Formalin	Water treatment	Finfish eggs Shrimp (Penaeid)	Antiprotozoal Antifungal
Human chorionic gonadotropin	Injection	Finfish (male and female broodstock)	Spawning aid
35% Hydrogen peroxide	Water treatment	Freshwater-reared Salmonids	Bacterial gill disease
35% Hydrogen peroxide	Water treatment	Freshwater-reared finfish eggs	Saprolegniasis
35% Hydrogen peroxide	Water treatment	Freshwater-reared cool water finfish and channel catfish	External columnaris disease
Oxytetracycline dihydrate	Premix (for feed)	Lobsters	Gaffkemia
Oxytetracycline dihydrate	Premix (for feed)	Salmonids	Ulcer diseases
Oxytetracycline dihydrate	Premix (for feed)	Salmonids and catfish	Bacterial hemorrhagic septicemia and *Pseudomonas* disease
Oxytetracycline hydrochloride	Water treatment	Finfish	Skeletal marking
Sulfadimethoxine ormetoprim	Premix (for feed)	Catfish Salmonids	Enteric septicemia of catfish Furunculosis
Sulfamerazine	Premix (for feed)	Trout	Furunculosis
Tricaine methanesulfonate	Water treatment	Fish	Temporary immobilization

[a] Listed indications are broad. For specific indications, the drug label or Code of Federal Regulations should be consulted.

out in the guide, it is unlikely that any action will be taken against them. In effect, this guide allows veterinarians to recommend approved medicated feeds to be fed to minor food-producing animals in an extralabel manner. For example, pheasants can be fed medicated feed that is labeled for chickens or turkeys. The CPG was revised in late 2016 to accommodate the change of medicated feeds containing antimicrobials important in human medicine from over-the-counter (OTC) to veterinary feed directive (VFD) marketing status. It provides a way for veterinarians to order such feeds even though VFDs do not permit legal extralabel use. The CPG is not the answer to the issue of drug availability, but is a temporary solution that should someday be made unnecessary by drug approvals. The CPG is available on the FDA website.

Minor Use and Minor Species Animal Health Act

In 2004, the Minor Use and Minor Species (MUMS) Animal Health Act was passed giving the FDA the

Table 51.5 Veterinary pharmaceuticals approved by the Food and Drug Administration as of May, 2014 for use in miscellaneous minor food-producing animals

Drug	Formulation	Species	Indication[a]
Lasalocid	Premix (for feed)	Rabbits	Coccidiosis
Lincomycin hydrochloride	Water soluble powder	Honey bees	American foulbrood
Oxytetracycline	Premix (for feed) Water soluble powder	Honey bees	American and European foulbrood
Sulfaquinoxaline	Premix (for feed)	Rabbits	Coccidiosis
Tricaine methanesulfonate	Water Treatment	Amphibians	Temporary immobilization
Tylosin tartrate	Water soluble powder	Honey bees	American foulbrood

[a] Listed indications are broad. For specific indications, the drug label or Code of Federal Regulations should be consulted.

opportunity to encourage the approval of drugs for minor animal species and for minor uses in major animal species. Among the provisions of the Act, the MUMS legislation modified the Federal Food, Drug and Cosmetic Act in three significant ways, by providing the options of conditional approval, indexing, and designation for veterinary drugs used in minor animal species (Public Law 108–282).

Conditional Approval

The MUMS Act provides an opportunity for drugs to be conditionally approved. This option is limited to drugs that will be used for minor uses in major species and for minor species, including minor food-producing animals. Conditional approval allows drug sponsors to market the product on a yearly renewal basis for up to 5 years while gathering effectiveness data. All other requirements for a conditional approval (e.g., human food safety) are the same as for a full approval. The standard for conditional approval is “a reasonable expectation of effectiveness” rather than “substantial evidence.” Within the 5 years allowed for conditional approval, the sponsor must provide effectiveness data to the full approval standard. Conditionally approved new animal drugs must be so labeled and are not eligible for extralabel use.

Indexing

The intent of the Legally Marketed Unapproved New Animal Drug Index is to provide a means for sponsors to make products available for markets that cannot meet requirements of the NADA due to small populations, intrinsic value of the animals, wide variety of species, etc. It is limited to minor species, not minor uses. Indexing does not apply to minor food-producing animals as it is reserved for drugs to treat diseases or conditions in animals where human food safety is not a concern. The index is intended to serve the needs of laboratory animals, zoological animals, companion birds, ornamental fish, etc.

Designation

The designation portion of the MUMS legislation created a program for veterinary medicine that largely mirrors what has been done with the orphan drug program in human medicine. Designation is intended to provide incentives to encourage pharmaceutical companies to support drug development for minor species and minor uses. Designated drugs are granted 7 years of exclusive marketing rights beginning on the date of approval or conditional approval. The MUMS Act also authorizes availability of grants to support safety and effectiveness testing of designated new animal drugs.

Programs Supporting the Use of Veterinary Pharmaceuticals in Minor Food-Producing Animals

Minor Use Animal Drug Program

As already noted, the small market size for therapeutic veterinary products for minor food-producing animals makes the time and expense of drug approval unattractive to pharmaceutical companies. To assist and increase drug availability for minor food-producing animals, the United States Department of Agriculture (USDA) sponsors a program to generate data to support such drug approvals.

The Minor Use Animal Drug Program, formerly known as the National Research Support Project #7 (NRSP-7), is a USDA program to support research into the safety and effectiveness of drugs for use in minor species and for minor uses in major species of agricultural importance. This encompasses animals used to produce food or fiber. This program is intended to help fill the need for safe and effective drugs for use in minor food-producing animals, such as sheep, goats, rabbits, game birds, bison, deer, honey bees, and aquaculture species. A review of this program has been published by Ringer et al. (1999). The program accepts requests for needed drugs from a wide range of stakeholders, including veterinarians, researchers, producers, and pharmaceutical companies. In most cases the program funds studies to complete data requirements for effectiveness, target animal safety, human food safety, and environmental safety. As these studies are reviewed and accepted by CVM, the data are gathered into a Public Master File, and made available on the FDA website. A pharmaceutical sponsor can use these data in conjunction with its own manufacturing and labeling information to support an NADA for the product. In this way, costs to the pharmaceutical company are drastically reduced.

Since the Minor Use Animal Drug Program has a limited budget, it generally, but not always, selects projects for minor food-producing animals that will be supplemental to existing products already labeled for use in major food-producing animals. The toxicology portion of the human food safety technical section of an NADA is much too expensive for the program to fund an original approval for a minor food-producing animal. If this work was already done by the pharmaceutical sponsor to support a cattle or swine approval, for example, it does not need to be repeated for the minor food-producing animal. Residue depletion and antimicrobial resistance issues, however, still need to be addressed. Effectiveness and target animal safety need to be demonstrated in the minor food-producing animals and the environmental component must be addressed. Again, if an approval for a major food-producing animal already exists, a categorical exclusion from the requirement to provide an environmental assessment is a likely option, unless the new approval is for use in aquaculture.

At any given time, the Minor Use Animal Drug Program is sponsoring three to five active projects. Working in cooperation with universities, producers, veterinarians, and government agencies, the program leverages a small budget into many studies. Since its inception in the early 1980s, the program has been successful in supporting over 29 drug approvals for a wide range of species. Information about the program and its projects can be found at: www.nrsp7.org.

There are also other entities that follow this basic model of providing public data that can be used to support drug approval for minor species, especially for aquaculture. Public entities such as the US Fish and Wildlife Service and the US Geological Survey conduct research in these areas. Their work has been of great value in the development of new products for managed aquatic stocks.

The Food Animal Residue Avoidance and Depletion Program

The Food Animal Residue Avoidance and Depletion Program (FARAD) assists veterinarians in the establishment of scientifically based withdrawal intervals (not withdrawal times) to prevent the occurrence of illegal residues in edible products derived from food-producing animals treated in an extralabel manner (Payne et al., 1999). A *withdrawal time* is a legal requirement established by the FDA/CVM as part of the drug approval process. A *withdrawal interval* is a recommended preslaughter or milk discard time provided by FARAD to veterinarians in support of the AMDUCA requirement for an extended withdrawal interval for extralabel drug use in food animals.

FARAD utilizes a decision tree approach to the problem and uses all available published data (and unpublished confidential data from pharmaceutical firms whenever possible) to guide the establishment of a withdrawal interval. When tissue or milk data are not available, the establishment of withdrawal intervals sometimes relies on blood (and serum or plasma) pharmacokinetic data to help define the final phase elimination of a drug. In such cases where serum or plasma data are used for estimating tissue residue depletion, it is assumed that the final edible tissue elimination phase will be at least as long as that seen in plasma. All possible data are used to help in this estimation, and whenever a recommendation is made, withdrawal estimations are increased to provide a wider margin of safety. In this way, “safety factors” are built in when assumptions must be made to help ensure that the estimated withdrawal interval will be sufficient. In many cases, data do not exist that can be used to make a withdrawal interval estimation, and the practitioner is advised to consider a different drug for treatment.

Methods for Estimating Withdrawal Intervals for Veterinary Pharmaceuticals Administered to Minor Food-Producing Animals in an Extralabel Manner

When drugs are used in an extralabel manner in food animals, veterinarians need to determine withdrawal intervals to prevent the occurrence of illegal drug residues. FARAD uses several methods to establish withdrawal intervals for drugs administered to minor food-producing animals in an extralabel fashion. All of the methods are dependent in some way on pharmacokinetic and tissue residue data from the minor and/or related major food-producing species. Techniques include comparative pharmacokinetics, allometric scaling, species grouping, and extrapolation based on in vitro or molecular data.

Comparative Pharmacokinetics

The pharmacokinetics of a drug specific to minor food-producing animals, including drug absorption, distribution, metabolism, and excretion, are the key processes determining withdrawal intervals for drugs used in an extralabel manner. These processes differ between species for many reasons, including the anatomy and physiology of the gastrointestinal tract, body composition and relative volumes of different tissues, the capacity and efficiency of different metabolic pathways, glomerular filtration, hepatic blood flow, and plasma protein binding (Lin, 1995; Toutain et al., 2010). When the pharmacokinetics of a drug are not completely known for a minor food-producing species, recommendations may need to be made by extrapolation from major food-producing species. When judging whether such extrapolation is feasible, it is important to consider the many factors that could result in differences in these pharmacokinetic processes between species.

One of the challenges of extrapolating withdrawal times from major to minor food-producing species is determining the appropriate target concentration of the marker residue (tolerance) for the minor species. The tolerance, which is the concentration of the marker residue in the target tissues that drives the withdrawal time in a particular species, is determined by the ratio of the marker residue to total drug residue. Although there may not always appear to be species differences in the types of metabolites formed, there may be significant differences in the ratios of these metabolites. For example, the metabolism of fenbendazole differs even among poultry species (Short et al., 1988); and cattle and sheep have been found to differ significantly in their capacity to metabolize xenobiotic substrates (Watkins et al., 1987). Species differences in metabolite ratios may therefore invalidate the practice of targeting a tolerance set for a major food animal species for a minor food animal species. To ensure safe residue concentrations, AMDUCA requires that if a tolerance is not established for the species or matrix in question, any residues will be considered violative. In food-producing animals, this means that the tolerance and consequently the necessary withdrawal interval may differ between major and minor food-producing animals. Juskevich published a review of comparative food animal drug metabolism that provides an excellent overview covering important factors that must be considered when comparing drug metabolism between species (Juskevich, 1987). Short (1994) published a review comparing factors that impact drug metabolism and disposition in sheep and other domestic ruminants to demonstrate whether pharmacokinetic profiles could be predicted for antimicrobials used in these animal species.

Classic Pharmacokinetic Studies

Studies that describe the pharmacokinetics of a drug in a small group of homogenous animals have been the cornerstone in the study of veterinary pharmacology. If such studies are conducted in more than one species, the results can be compared to inform interspecies differences in drug disposition. Thousands of manuscripts have been published on the pharmacokinetics of drugs in food-producing animals. Some of the studies focus on just one species whereas others are more helpful in that they compare the pharmacokinetics of drugs between major and minor food-producing animals (Modric et al., 1998; Craigmill et al., 2000; Lanusse, 2003; Mestorino et al., 2008; Baert and De Backer, 2003; Taggart et al., 2007). Chapter 3 of this text should also be consulted for an introduction to pharmacokinetic principles.

FARAD has been collecting pharmacokinetic data from the published literature since 1982. The FARAD database contains citations that contain pharmacokinetic data on chemical or drug residues for a variety of animal species. The data from numerous food-producing animals have previously been compiled and published as a reference textbook (Craigmill et al., 2006). In comparison to the major food-producing animals, the minor food-producing animals have considerably fewer published pharmacokinetic studies for the various categories of antibiotics, anthelmintics, nonsteroidal antiinflammatories, and exogenous hormones. Using ruminants as an example, Table 51.6 demonstrates the preponderance of pharmacokinetic-based publications in the FARAD database for cattle versus some of the minor food-producing animals. While the focus of the FARAD data collection has been residue avoidance, considerable data are also available on serum/plasma pharmacokinetics of drugs that can be useful for therapeutic purposes as well. Reviews on the relationship of pharmacokinetic studies to drug residues have previously been published (Lees and Toutain, 2011, 2012)

The limitation of these traditional pharmacokinetic studies, which are carried out in a small group of animals specifically selected for homogeneity, is that they do not provide an accurate reflection of the variability of drug disposition in the target population. Tissue residue studies are typically carried out in a similar manner, with a small number of animals serially slaughtered at various times after drug treatment allowing the establishment of the kinetics of residue depletion. It would be cost-prohibitive to conduct the full pharmacokinetic studies in larger numbers of animals needed to better understand population variability. Fortunately, nonlinear mixed effects models for population pharmacokinetics and metaanalyses provides a tool for pooling sparse data available from different sources to describe the variability in the population, and identify factors that contribute to this variability.

Population Pharmacokinetics and MetaAnalysis

One of the limitations of most comparative pharmacokinetic studies that have been done to date is the small number of animals of each species used. In most studies, all of the animals representing a species have been obtained from one supplier and are of one breed or strain. Therefore, the variability in parameters within a species (intraspecific variability) cannot be assessed. Such unknown variability within a species makes it even more difficult to evaluate variability between species (interspecies). Nonlinear mixed effects models can be used to study population pharmacokinetics and help to define this variability, but these methods require substantial amounts of data to give the best results. Fortunately, the method does not require a rich dataset for each animal in the population, and data can be pooled from various different sources, which makes it feasible to collect the needed data.

Martin-Jimenez and Riviere defined population pharmacokinetics as “a study of the basic features of drug disposition in a population, accounting for the influence of diverse pathophysiological factors on pharmacokinetics, and explicitly estimating the magnitude of the interindividual and intraindividual variability” (Martin-Jimenez and Riviere, 1998). An advantage to this technique is that

Table 51.6 Comparison of numbers of Food Animal Residue Avoidance Databank (FARAD) publications with drug concentration versus time dependent data for select food animal species as of July, 2017. Food animal species were considered for both United States of America and international markets.

Drug class	Chicken	Turkey	Cattle	Buffalo	Goats	Sheep	Rabbits	Swine	Equine	Cervids	Total
Aminoglycosides	166	24	568	19	63	146	163	114	161	2	1426
Anthelmintics	163	26	1321	105	332	1506	96	351	162	58	4120
Cephalosporins	40	6	515	40	109	85	310	176	130	4	1415
Fluoroquinolones	498	64	437	71	160	136	132	288	117	0	1903
Hormones	43	2	779	0	35	104	171	54	116	0	1304
Macrolides	187	18	495	3	69	123	47	225	68	10	1245
NSAIDs	95	28	504	16	139	109	166	154	523	0	1734
Penicillins/Beta-lactams	168	26	1418	39	98	177	320	332	289	0	2867
Sulfonamides	626	46	1009	87	270	225	187	617	160	0	3227
Tetracyclines	457	93	852	23	93	147	67	411	39	2	2184

it allows for assessment of how clinical factors (age, gender, renal and hepatic function, etc.) impact the pharmacokinetic parameter estimate. It can also be conducted using a multicompartment model to predict residue concentrations in specific tissues. This technique has been used to look at the pharmacokinetics of penicillin in the major food animal species cattle and swine (Li et al., 2014). The application of such a methodology would greatly benefit minor food-producing animals because collective sampling might ultimately establish significant baseline data reflecting the pharmacokinetic parameters of a drug in a field or production setting.

The nonlinear mixed effects approach can also be used to conduct metaanalyses, in which data from multiple published studies are carefully combined to gain a better understanding of a drug's pharmacokinetic variability in the larger population. Species can be included in these models as a categorical covariate explaining some of the observed variability. This approach was applied to procaine penicillin G in cattle and swine (Li et al., 2014) and oxytetracycline nonlactating cattle using FARAD data (Craigmill et al., 2004), as well as ampicillin trihydrate in cattle (Gehring et al., 2005) and flunixin in cattle (Wu et al., 2013). Metaanalysis requires careful selection of data according to predetermined standards to ensure data quality, including such factors as analytical methodology and study design. Under optimal conditions, the metaanalysis is conducted using population pharmacokinetic modeling procedures when individual animal pharmacokinetic data from multiple studies and individual animal covariate data (sex, weight, age, health, etc.) are available.

Physiologically Based Pharmacokinetic (PBPK) Modeling

Physiologically based pharmacokinetic (PBPK) modeling is a very effective method for making interspecies comparisons for drugs. Physiologically based pharmacokinetics offers another way to study interspecies relationships, and has been used extensively for predicting human tissue concentrations of toxicants using animal data. PBPK also offers researchers the opportunity to utilize in vitro metabolism data (K_m and V_{max}) to help predict clearance rates of chemicals in multiple species. PBPK modeling uses physiological and biochemical parameters, therefore this method can be mechanistic as well as predictive. Models can be adapted for different species by changing the physiological parameters accordingly. Physiological parameters used in PBPK models may be measured directly or derived from allometric equations. PBPK models have been developed for fish (Law, 1999), avian species including turkeys, chickens, pheasant, and quail (Pollet et al., 1985; Cortright et al., 2009), cattle (Achenbach et al., 1998; Leavens et al., 2014), horses (Trachsel et al., 2004; Knobloch et al., 2006), swine (Buur et al., 2006; Yang et al., 2012, 2013), rabbits (Tsuji et al., 1985), sheep (Craigmill, 2003), goats (Leavens et al., 2012), and bovine intramammary treatments (Whittem et al., 2012). A model for multiple species (cattle and humans) and routes of administration (oral and intramuscular) has also been developed (Lin et al., 2015). PBPK modeling has been used to evaluate oxytetracycline residues in cattle, sheep, and fish and may prove useful for predicting the depletion of drug residues in the tissues of other minor food-producing animals in the future (Achenbach et al., 1998; Law, 1999; Craigmill, 2003).

Allometric Scaling

For decades, veterinary medicine has used allometric scaling to calculate effective doses and dosage regimens for a variety of animals. For a detailed presentation of the use of allometry for interspecies pharmacokinetic parameter scaling, readers are referred to previously published review manuscripts (Huang and Riviere, 2014; Mahmood et al., 2006; Martinez et al., 2006). Riviere and colleagues (1997) gathered pharmacokinetic parameter data for 44 drugs using the Food Animal Residue Avoidance Depletion program and found that 11 of the 44 drugs showed significant allometric relationships of their pharmacokinetic parameters for extrapolation between veterinary species. Nine of these 11 drugs were antibiotics. Some of the drugs that did not demonstrate allometric relationships with respect to their pharmacokinetic parameters were low hepatic excretion drugs. For the other drugs, the results were equivocal. When this work was recently expanded to 85 drugs, it was found that body weight was significantly correlated with total body clearance and volume of distribution in 77% and 88% of the drugs, respectively (Huang et al., 2015). In this latter study, 12 drugs were identified to have at least one outlier species for clearance and 10 drugs for volume of distribution at steady state.

These pharmacokinetic allometric studies can be used to estimate withdrawal intervals using kinetic parameter data for drugs in blood, serum, or plasma. For unmetabolized drugs (e.g., ones that are not highly protein bound in plasma and are not specifically bound by tissue components), one could expect that tissue residue parameters would mirror blood kinetic parameters. Tissue residue elimination would then be dependent on blood flow and the tissue/plasma partition coefficient. Drugs that are highly fat soluble would be removed from fat depots slowly. This slow removal would subsequently be seen as a prolonged elimination phase.

Species Grouping

Some researchers, including some in the Minor Use Animal Drug program, have been working collaboratively to develop approaches to group minor food-producing animals. This is explored by doing studies in an “indicator” species for each group. The primary focus of this effort has been on aquaculture species. This is due to the large number of aquatic species that are in need of approved drugs to treat diseases and parasites that are often caused by the same organism in multiple species. Several possible ways to achieve this goal include grouping of species based on physiology, drug delivery mechanism, target pathogen, culture system, economic importance, and human food safety concerns (Greenlees and Bell, 1998). Sources of variability in the pharmacokinetics of drugs in aquaculture species include interspecific variability, intraspecific variability, habitat variability, temperature, size/age, and sexual maturity (Gingerich et al., 1998). Poikilothermic animals present a greater challenge than homeothermic animals because the environmental temperature has such a great effect on their physiology. Therefore, studies focusing on poikilothermic animals need to consider environmental temperature as a mixed “physiological” and environmental variable. The FDA CVM accepts some species grouping models, mainly for aquaculture, for new animal drug applications. See Guidance for Industry #61 on the FDA website.

Future Directions: In Vitro and Molecular Studies

In vitro and molecular based research might help establish some differences in drug metabolism before going to whole animal models. In vitro methods for exploring comparative metabolism include P450 assays, microsomes, tissue slices, isolated cells, and cell culture. P450 assays have been used to evaluate species differences for major and minor food-producing animals (Dalvi et al., 1987; Nebbia et al., 2001; Machala et al., 2003; Dacasto et al., 2005). Microsomes from food-producing animals have been used to evaluate gender and breed differences (Dacasto et al., 2005). Another study evaluated the biotransformation of cultured hepatocytes from goats, sheep, and cattle using five different test substrates and determined that cultured hepatocytes appeared to be a good in vitro model to study comparative metabolism of veterinary drugs in these animal species (van ’t Klooster et al., 1994). Reviews of P450 metabolism in veterinary species have been published (Fink-Gremmels, 2008; Antonovic and Martinez, 2011).

Genomic and proteomic methods to evaluate interspecies differences in drug metabolizing enzymes might also be useful for minor food-producing animals. Chapter 50 of this textbook introduces the discipline of pharmacogenomics applied to veterinary species. Examination of DNA using Southern blot analysis can be used to determine if genes exist that code for specific drug metabolizing enzymes. Northern blots may be used to find mRNA transcripts of genes that code for drug metabolism enzymes. For minor food-producing animals, this investigation could start by using cDNA probes for a subfamily of P450 enzymes that are extensively involved in drug metabolism, such as the CYP3 family. Hepatic mRNA from a minor food-producing animal of interest could also be evaluated using commercially available P450 cDNA probes. Using this approach, one could establish only that probe hybridization patterns were similar between the minor food-producing animals being considered. If similar hybridization patterns existed, one could hypothesize that those species evaluated might be similar in drug metabolic capability, at least on a qualitative basis. This hypothesis could then be tested using in vitro (including expression vectors and drug substrate assays) or in vivo methods.

Immunoblotting would be another approach to assess similarities or differences between minor food-producing animals. For this approach, antibodies for the specific protein of interest would need to be developed for minor food-producing animals because the commercial probes/antibodies are usually from nonfood-producing animals (e.g., mouse, rat, monkey, human). Therefore, the use of the commercial antibodies in such assays would be limited for interspecies analysis because the metabolic enzymes from different species cannot be assumed identical. In addition, structural homology does not guarantee functional homology. Nevertheless, because there is considerable sequence conservation across species in the cytochrome P450 enzyme superfamily and metabolic enzymes in general, such studies may be quite useful to characterize potential metabolic enzyme capacities of minor food-producing animals.

As the field of proteomics expands and protein sequence methods become quicker, the identification and characterization of drug-metabolizing enzymes for minor food-producing animals will be easier and more economical. Progress in this area is dependent on increased funding for such research, which may result from growing concern over therapeutic drug residues in food-producing animals.

Summary

Improving drug availability for food-producing minor species is proceeding on several fronts. There has been continual growth in the use of available incentive and research programs. It will take time for these to have a more significant effect. With the great variety of diseases and species that this group represents, every possible incentive must be exploited and every research option explored. This will undoubtedly require that funding for the grants and research programs be increased. Veterinarians, researchers, pharmaceutical companies, government agencies, producers, and consumers all have a stake in the success of this work.

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52

Unique Considerations Pertaining to the Use of Drugs in Food Animals

Michael D. Apley

Introduction

Veterinarians often focus on an area of veterinary medicine and become competent in the pharmacology of drugs associated with that area. As either a veterinarian or a clinical pharmacologist working with food animals, there are additional considerations beyond the animal–drug interaction.

When using drugs in food animals, there must be a consideration of the effect of the drug on the entire population of animals within the production environment, and also within the ultimate supply chain for human consumption. Addressing the safety of meat, milk, and eggs produced by modern agriculture not only involves the evaluation of safety of drug residues, but also other effects such as environmental safety, and selection for resistant bacterial pathogens. The safety of drugs in food animals is no longer only closely observed by regulatory and legislative bodies, but now also by food distribution/retail chains as well as their customers, the consumer. At the time of this writing, due to evolving food retailer policies, we are coming into a period where the effects of social media trends and marketing studies may supersede regulatory and legislative pressures as a major driver in determining what drugs are used in food animals.

The use of drugs in animals intended for use as food primarily involves a subset of veterinary drugs focused on prevention, control, and treatment of infection (both bacterial and parasitic), inflammation, analgesia, anesthesia, reproduction, and performance enhancement. Specific characteristics of these drugs are described elsewhere in this book. This chapter summarizes drugs that should be familiar to a veterinarian involved with food animals, and also describes special considerations related to these drugs when used in food animals.

The lists of drugs and drug groups presented in this chapter are US centric. There are other drugs used around the world in food animals, and perhaps other drug groups. These lists are presented as a means of general appreciation of the breadth of drugs widely employed in food animal production along with considerations for use unique to food animal production; representations of approval or lack of approval, availability, or legality in specific countries are neither intended nor implied.

Extralabel Use

The legality of the use of a drug in food animals in an extralabel manner is dependent on the country in which the drug is used. An example of a cross-border difference is the extralabel use (ELDU) of drugs in the feed of food animals in the USA and Canada. Drugs may be used in feed in an extralabel manner in Canada, but this use is prohibited for food animals in the USA (Health Canada, 2015) An exception in the USA is the allowance for ELDU under regulatory discretion of drugs in the feed for minor food animal species, such as sheep and goats (FDA, 2016). This Compliance Policy Guide (CPG) states that enforcement discretion will be exercised provided that certain requirements are met for ELDU in the feed of minor species.

The regulations for ELDU in food animals in the USA were promulgated and finalized in 1996 to codify the Animal Medicinal Drug Use Clarification Act (AMDUCA) of 1994 (FDA, 1996). Under the regulations, ELDU is "limited to treatment modalities when the health of an animal is threatened or suffering or death may result from failure to treat." This precludes ELDU for production purposes; examples are unapproved estrous synchronization protocols, lactation induction protocols, and extralabel use of growth-promoting hormonal implants. Specific ELDU considerations related to some drug classes are discussed below. It is also important that the veterinarian know the list of drugs for which ELDU in food animals is specifically prohibited. As of this writing, the most current list contains the following drugs (FDA, 2015a).

Under the AMDUCA provisions, FDA has the right to prohibit extralabel uses of certain drugs in animals. The following drugs (both human and animal), families of drugs, and substances are prohibited from extralabel uses

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in all food-producing animals, including horses intended for human food:

- Chloramphenicol
- Clenbuterol
- Diethylstilbestrol (DES)
- Dimetridazole
- Ipronidazole and other nitroimidazoles
- Furazolidone and nitrofurazone
- Sulfonamide drugs in lactating dairy cattle, except for the approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine
- Fluoroquinolones
- Glycopeptides
- Phenylbutazone in female dairy cattle 20 months of age or older
- Cephalosporins (not including cephapirin) in cattle, swine, chickens, or turkeys:
  - For disease prevention purposes;
  - At unapproved doses, frequencies, durations, or routes of administration; or
  - If the drug is not approved for that species and production class.

The following drugs, or classes of drugs, that are approved for treating or preventing influenza A are prohibited from extralabel uses in chickens, turkeys, and ducks:

- Adamantane
- Neuraminidase inhibitors

The above list can be found at Title 21 of the CFR, Part 530.41. Currently, no approved drugs are prohibited from extralabel uses in companion animals.

The list of prohibited drugs in the country in which a veterinarian is practicing, or to which food products are being exported, should be well understood. In the United States, it is important to understand that a lactating dairy cow and a dairy cow 20 months of age or older are the same animal for regulatory purposes; the fact that a dairy cow greater than 20 months of age is in the dry period does not change her status as a lactating dairy cow.

An area of ELDU that receives special attention in regulations is the preparation of compounded drug products. In the USA, the use of compounded drugs in food animals had been the subject of compliance policy guide (CPG) Section 608.400 in addition to inclusion in the AMDUCA regulations (FDA, 1996). While the AMDUCA regulations remain unchanged, CPG 608.400 was withdrawn May 19, 2015, and a new draft Guidance for Industry (GFI #230) was released related to compounding from bulk substances. Guidance for Industry #230 addresses compounding from bulk substances in food animals only in that this is not allowed (FDA, 2015b). At the time of this writing the FDA/CVM has asked for input on a list of drugs for which compounding from bulk substances may be allowed in companion animals, with the subject of compounding drugs such as antidotes for food animals from bulk substances remaining unaddressed.

Bulk substances are drug forms which are not the subject of a final New Animal Drug Application (NADA) or New Drug Application (NDA) approval. Compounding in the USA is a very contentious issue as some compounding pharmacies and veterinarians are able to make substantial margins on selling unapproved drug compounds. There are potential downfalls of compounded products, whether from bulk substances or approved drugs, of which the prescribing (or selling) veterinarian should be aware. These include sterility and presence of endotoxin for injectable products, purity, potency, toxicity, violative residues, instability of the final compounded product, and potential lack of efficacy due to insufficient bioavailability. Another issue with compounded products specific to food animals is uncertainty as to the withdrawal time to assure that no violative residues enter the food chain. A compounded product should in no way be confused with a generic product; the latter has an approved FDA label and is subject to good manufacturing processes, which are inspected by the FDA.

The issue of compounding of drugs for use in food animals is a good example of regulatory flux, and that veterinarians must be ever vigilant for regulatory changes.

Residues and Withdrawal Times

The foremost additional responsibility of using drugs in food animals is to protect the safety of the food products from the treated animal(s) by assuring that violative residues do not enter the food chain. An overview of the regulatory process related to residues in animal food products may be found in Food and Drug Administration Center for Veterinary Medicine (FDA/CVM) Guidance for Industry (GFI) #3 (FDA, 2006) and in Chapters 55 and 61 of the present text. The classic approach for determining a slaughter withdrawal time in the USA has been to first determine the toxicity of the compound in laboratory animals by establishing a No Observable Effect Level (NOEL). The NOEL is combined with the regulatory average human weight and a safety factor to establish a total Acceptable Daily Intake (ADI) for the drug residues over a human lifetime. A safe concentration in edible tissues is then calculated by estimating daily intake of muscle tissue, liver, kidney, and fat and using the ADI to establish the residue concentration that may be present in each tissue based on intake. In addition, the presence of residues in milk and eggs are considered for appropriate production classes; if the drug is to be used in an animal producing milk or eggs, the ADI must be partitioned between a serving of muscle tissue, liver, kidney, or fat and consumption of milk or eggs, which may also contain the same residue. In the USA, a tolerance addressing a single marker residue is then developed for the target residue organ (and possibly for other tissues, including milk and eggs if appropriate). In other countries a Maximum Residue Level (MRL) is established, which may be for one residue or a group of residues from the drug. Finally, a withdrawal time for label use of the product is established based on evaluating the MRL/tolerance in light of a study determining depletion of the drug from edible tissues in the target species, and applying statistical methods to these data.

Along with the process of establishing a slaughter withdrawal time, the sponsor must develop and validate an assay for detection of marker drug residue(s) in the target residue organ Other countries may have differing requirements for the process of establishing a withdrawal time for food animal drugs.

In the USA, as in some other countries, the extralabel use of drugs in food animals is allowed when used in accordance with the Animal Medicinal Drug Use Clarification Act (AMDUCA) regulations (FDA, 1996). In these cases, the veterinarian is responsible for assuring that no violative residues enter the food chain and is required to assign an exaggerated slaughter withdrawal time. In cases where the drug is approved for use in that species, during the approval process it was required to establish a tolerance, regulatory method to detect residues, and withdrawal time for that approval. Therefore, the veterinarian is often able to minimally extend the withdrawal time if the label regimen is used. If there is no approval in that species, or an altered regimen from that on the label is used, then the veterinarian must find information to support the assignment of an exaggerated slaughter withdrawal time, or else the animal must not enter the food chain. For the USA, and some other countries, the veterinarian has access to the Food Animal Residue Avoidance Databank (FARAD, or gFARAD for the global version) to aid in this process (FARAD, 2015). If information is not able to be found to assure that no violative residues will occur, the treated animal must not enter the food chain.

The lack of a tolerance for a drug used in food animals requires full attention to potential residues. The presence or absence of a tolerance may be evaluated in the USA by accessing 21 CFR part 556 (FDA, 2015c). Other countries or unions may have their own lists, or adopt standards such as Codex Alimentarius (CODEX, 2013). If there is no tolerance for the species in which the drug is being used, then the concentration that can be detected by the most sensitive assay essentially becomes the tolerance, which historically has continued to decline as analytical technology advances. Estimating slaughter withdrawal times by extrapolating beyond available data down to “zero” may be hampered by the fact that drug elimination from edible tissue may be very different at concentrations lower than concentrations observed in available data. This topic is further discussed in Chapter 61.

Another common challenge in avoiding violative residues is the difference in tolerances and/or MRLs between exporting and importing countries. If a food animal product originates in the USA, use of drugs in production settings must take into account whether the food product(s) might be exported to Russia, the European Union, Japan, China, or South Korea, as examples. In some cases the difference may be as extreme as the US tolerance for total residues of tetracyclines (e.g., chlortetracycline, oxytetracycline, and tetracycline) of 2000 ppb compared to no tolerance or an MRL of 10 ppb in an importing country.

There are some specific cases in the USA where a lapse of residue awareness can cause significant regulatory issues for a veterinarian and their client(s). The first example is that gentamicin has an approval in cattle for topical use in the eye for infectious bovine keratoconjunctivitis. However, during the approval process, no systemic concentrations were detected after the label regimen and therefore no tolerance was required in edible tissues. It has been demonstrated that gentamicin has prolonged depletion characteristics in the bovine kidney after extralabel systemic administration, which when combined with the absence of an acceptable concentration at slaughter, sets up a drastically prolonged required ELDU withdrawal time of approximately 18 months in cattle.

Another example is the use of penicillin G in swine. Penicillin G is approved for swine; however, the tolerance for swine currently listed in 21CFR Part 556 is zero. The sensitivity of the Kidney Inhibition Swab (KIS) test (35 ppb) and the follow-up Food Safety and Inspection Service (FSIS) mass spectrometery test (25 ppb) have resulted in an issue with violative residues in sows if a sufficient exaggerated withdrawal time has not been used. A tolerance of 50 ppb (negligible residue) in the uncooked edible tissues of cattle has been established.

Alteration of the injection site while observing the rest of the label regimen may be sufficient to drastically alter the slaughter withdrawal requirements. Ceftiofur crystalline free acid is labeled for administration to cattle in either the middle third of the ear or at the base of the ear. Some veterinarians have advised producers to inject this product subcutaneously in the neck for convenience. However, the altered regimen results in a significantly prolonged residue profile, contributing to increased risk for violative residues in addition to unproven efficacy by this route. This is an example of the attending veterinarian not thinking through all the ramifications of ELDU.

The issue of extralabel use of florfenicol in lactating dairy cattle illustrates that the use class of the treated food animal on the product label may be as important as the species. Examination of the tolerances in 21 CFR part 556 reveals a tolerance of 3700 ppb for florfenicol in the liver of cattle. However, lactating dairy cattle are not included on the label as a use class, resulting in an interpretation of no tolerance for florfenicol in edible tissues of a lactating dairy cow. This results in an extremely prolonged slaughter withdrawal time requirement for extralabel use in a lactating dairy cow as opposed to a beef animal. A lactating dairy cow for regulatory purposes is a dairy cow 20 months of age or older, regardless of lactation status. In addition, there is no tolerance for florfenicol in milk, resulting in a prolonged milk discard time. Florfenicol use in lactating dairy cows was highlighted in the 2012 FDA milk residue study; although only 15 violative residues were found out of 1912 samples (0.78%), 10 of these violative residues were florfenicol (FDA, 2015d). Other antibiotics for which this is an issue in lactating dairy cows include tilmicosin, gamithromycin, and tildipirosin.

Antibiotics

Here the term "antibiotics" is used according to the societal use, to include antimicrobials. The relationship of the terms antibiotic and antimicrobial is sufficiently complicated to have merited entire book chapters (Bennett, 2015). While this may at first seem a case of trivial semantics, the subject is actually quite important for products marketed with claims such as "antibiotic free". For example, would sulfonamides and fluoroquinolones be allowed in an animal-derived product which is promoted as antibiotic free? Yes, according to the most pedantic interpretation of the terms because they are by definition antimicrobials and not antibiotics, but in practical usage, no.

Table 52.1 summarizes US food animal antibiotic approvals with approved routes for swine, beef cattle, nonlactating dairy cattle, goats, sheep, chickens, and turkeys. Table 52.1 is US centric but represents classes of antibiotics approved throughout the world. Appearance in Table 52.1 does not guarantee that the antibiotic is currently marketed in the USA. Examples of antibiotics considered important for veterinary use around the world may be viewed in the World Association for Animal Health (OIE) list of antimicrobials of veterinary importance (OIE, 2017). The US approval list in Table 52.1 and the OIE list are very similar as far as the classes cited, with additional class members in use outside of the USA.

Table 52.1 was constructed by reviewing the on-line database for the US FDA *Green Book* at the website Animal Drugs @ FDA (FDA, 2014). All drugs discussed in this chapter may be evaluated for label information at this website. The New Animal Drug Approval Number (NADA) may then be used to access the FDA/CVM Freedom of Information (FOI) Summary database (FDA, 2015e). The FOI summaries provide information for studies conducted during the approval process, including substantial evidence of efficacy, pharmacokinetic studies, microbiology, target animal safety, user safety and areas of human food safety, including toxicology, residue chemistry, microbial food safety, and the analytical method for residues.

A classification of antibiotic importance to human medicine has been put forth by the FDA/CVM in Appendix A of Guidance for Industry (GFI) #152, and also by the World Health Organization (WHO) (FDA, 2003; WHO, 2011). Antibiotics reported in Table 52.1 are divided by their status as being listed or not listed as medically important for human medicine in the USA. The WHO list of medically important antibiotics would closely resemble the FDA/CVM list, with the addition of some classes such as the pleuromutilins. The WHO also classifies four critically important antibiotic groups as being of highest priority; these are the fluoroquinolones, third and fourth-generation cephalosporins, macrolides, and the glycopeptides. It is important to note that the FDA/CVM and WHO antibiotic classifications are in reference to importance to human medicine, and are not based on the potential for use in food animals to select for resistance in human pathogens.

The routes of administration for medically important antibiotics in Table 52.1 are only listed for their prevention, control, or therapeutic claims. A prevention claim is considered to be when no clinical cases are yet apparent but clinical experience indicates that a disease challenge is imminent. A control situation is when clinical cases have been detected in a population and the antibiotic is administered to both clinically diseased and clinically normal animals in an attempt to stop the further spread of the outbreak. The discussion of these uses has expanded to include discussion of "routine" prevention and control, indicating routine application of these treatment strategies rather than in specific instances based on evaluation of individual animal groups.

More emphasis is being put on the amount of antibiotics being used in food animals. In the USA, the Animal Drug User Fee Act (ADUFA) requires that the FDA/CVM collect and report annual sales of antibiotics with labels that include food animal uses. The relative sales of antibiotics approved for food animals in the USA, as classified by weight of active compound, and reported for the year 2013 are given in Table 52.1. From these data, it is evident that the tetracycline class dominates sales of active compound in food animals in the USA, as opposed to the fluoroquinolones and cephalosporins which comprise a small proportion. An estimate of in-feed use of antibiotics in swine production in the USA based on 2006 data also found that the tetracycline class comprised

Table 52.1 Antibiotics labeled for food animal use in the United States classified by medical importance status with reported sales proportion in 2013, by species and labeled routes of administration. These routes may be as part of a combination product, are approved specific to disease indications, and may also include only specific use classes and age restrictions within the species. This table contains label approvals as represented at *Animal Drugs@FDA*, the electronic version of the *Green Book* containing FDA CVM approvals.

Medically important antimicrobials as defined in Guidance 152 Appendix A

% of antibiotic sales for food animals in USA for this category in 2013	Class	Drug	Swine	Beef cattle, nonlactating dairy cattle	Lactating dairy cattle	Goats	Sheep	Chickens	Turkeys
2.9%	Aminoglycosides	Dihydrostreptomycin			IMM				
		Streptomycin	O	O	IMM			O	
		Gentamicin	W, I, O	T				I	I
		Neomycin	W,M,F,O	W,M,F,O		W,M,F,O	W,M,F,O	W,M,F	W,M,F
		Spectinomycin	O					W, I	I
0.3%	Cephalosporins	Ceftiofur	I	I	I, IMM	I	I	I	I
		Cephapirin			IMM				
0.2%	Fluoroquinolones	Enrofloxacin	I	I	I				
		Danofloxacin		I					
2.6%	Lincosamides	Lincomycin	I,F,W					W,F	
		Pirlimycin			IMM				
6.1%	Macrolides	Tulathromycin	I	I					
		Erythromycin		F,I	IMM			W,F	F
		Gamithromycin		I					
		Tildipirosin		I					
		Tilmicosin	O	O,I					
		Tylosin	F,I,W	F,I				F,W	W
		Tylvalosin	W						
9.0%	Penicillins	Amoxicillin			IMM				
		Ampicillin	O,W,I	O,I	I				
		Cloxacillin			IMM				
		Hetacillin			IMM				
		Penicillin G	I	I	IMM				W

4.2%	Sulfonamides	Sulfadimethoxine		O,W,I	I			W	W
		Sulfadimethoxine/ Ormetoprim						F	F
		Sulfamethazine	W,F	W,F,O				W	W
		Sulfachlorpyridazine	W,O	W,O,I					
		Sulfaethoxypyridazine	W	W,O,I					
		Sulfamerazine						W	W
		Sulfaquinoxaline		W				F,W	F,W
70.8%	Tetracyclines	Chlortetracycline	W,F,O	W,F,O			F	W,F	W,F
		Oxytetracycline	W,I,F	W,I,F,O	I		W,F	W,I,F	W,I,F
		Tetracycline	W	W				W	W
Not Individually Reported = 3.9%	Amphenicols	Florfenicol	W,F	I					
	Streptogramins	Virginiamycin	F	F				F	F
Antimicrobials not categorized as medically important as defined in Guidance 152 Appendix A									
79.3%	Polyether Ionophores	Monensin		F		F			F
		Lasalocid		F			F	F	F
		Laidlomycin		F					
		Salinomycin						F	
		Narasin	F					F	F
20.7%	Aminocoumarins	Novobiocin			IMM				
	Glycolipids	Bambermycins	F					F	F
	Polypeptides	Bacitracin zinc	F	F				F	F
		Bacitracin Methylene Disalicylate	F	F				F	F
	Pleuromutilins	Tiamulin	W,F						
	Quinoxaline derivatives	Carbadox	F						

F, feed; IMM, intramammary; I, injectable; IU, intrauterine; M, milk; O, oral; S, solid dose implant; T, topical; V, intravaginal; W, water.

approximately two-thirds of total use as classified by weight of active compound (Apley et al., 2012).

Antibiotic sales data for 2012 are also available for 26 countries in the European Union, as reported in the European Medicines Agency (EMA) European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) (EMA, 2014). These data are reported in a different manner than for the USA, adjusted with a "population conversion unit," which adjusts the kg of active compound sold in relation to kg weight of animals (either as populations in the country, transported, or slaughtered) at the estimated time of treatment. In this report, horses are included as food animals. Proportion of all sales for the antibiotics listed as the highest priority in human medicine by the WHO (with ranges across EU reporting countries) were 0.2% (0.01% to 1.2%) for third and fourth-generation cephalosporins, 1.7% (0.02% to 11%) for fluoroquinolones, and 8% (0.04% to 17%) for macrolides. Anticoccidials such as the ionophores are not classified as antimicrobials in the European Union, so are not included in these data. The dominant classes reported in the ESVAC report were the tetracyclines (37%), penicillins (22%), sulfonamides (10%), and macrolides (8%). Although caution must be exercised in comparing these data to the US proportions presented in Table 52.1 due to differences in reporting methods, it appears that the dominant classes of antibiotics used in food animals in the EU mirror those used in the USA

There is an important caveat in that sales data are not necessarily an accurate indication of how and where antibiotics are being used in food animals. A study evaluating sales versus actual end-user data in Denmark and the Netherlands confirmed that detailed use data is needed to define how and where antibiotics are being used in food animals (Bondt et al., 2013).

The concerns about antibiotic use in food animals may be collectively addressed with the term "microbial safety". The most meaningful distinction in the consideration of microbial safety is whether or not the class of antibiotics is classified as important to human medicine. The second critical consideration is if nonmedically important antibiotics may coselect for resistance to medically important antibiotics when used in food animal production systems.

Areas of the world have progressed at different rates in evaluating and addressing concerns about resistance in human pathogens related to use of antibiotics in food animals. Prohibition of antibiotics for growth promotion started in Europe with Sweden in 1986. Denmark banned growth promotion use of avoparcin in 1995 and virginiamycin in 1998. The use of all antibiotics for growth promotion in Denmark, except in pigs up to 35 kg live weight, were removed due to a voluntary stop in 1998. The last use of antibiotic growth promoters in Danish pigs up to 35 kg live weight were phased out in the fourth quarter of 1999 (DANMAP, 2010).

The precautionary principle has been used as a guide in addressing antibiotic growth promoters in the EU (EU, 2007) "The precautionary principle enables rapid response in the face of a possible danger to human, animal or plant health, or to protect the environment. In particular, where scientific data do not permit a complete evaluation of the risk, recourse to this principle may, for example, be used to stop distribution or order withdrawal from the market of products likely to be hazardous."(EU, 2011).

Based on this principle, the EU banned the use of avoparcin for growth promotion in 1997, and then in 1999 also banned bacitracin, spiramycin, tylosin, and virginiamycin for this use (Aerestrup et al., 2010). Anticoccidials are not captured in the antimicrobial use estimates in the EU; they are classified as anticoccidial feed additives and their use for this purpose continues to be allowed. The EU removed the final four antibiotics from approval for growth promotion in 2006; these were monensin and salinomycin (ionophores), avilamycin (an oligosaccharide), and flavophospholipol (bambermycins, seen as a glycolipid in Table 52.1). None of these compounds are classified as medically important in the USA; nonmedically important antibiotics such as the ionophores and the bambermycins will continue to be allowed for growth promotion use in the USA after the implementation of GFI #209 in 2016.

In the USA, concerns led to GFI Document #209 (2012) which called for voluntary compliance in removing growth promotion claims from labels of medically important antibiotics and also adding the requirement for veterinary supervision or oversight in the uses of these antibiotics in the feed or water for prevention, control, or therapy of diseases in food animals (FDA, 2012a). All 26 sponsoring companies, representing 283 labels, agreed to comply with GFI 209. The FDA/CVM followed GFI 209, with GFI #213, and fully implemented the recommendations on January 1, 2017. As of that date, all medically important antibiotics added to feed and water will require veterinary oversight and a written order compliant with the Veterinary Feed Directive (VFD). The VFD requirements are discussed in more detail in Chapter 59.

The microbial safety of antibiotics to be used in food animals in the USA is addressed during the approval process through two main components, the first a qualitative risk assessment described in GFI #152. In this process, rankings of high, medium, or low are determined for the release of a resistant organism (*Salmonella* or *E. coli*) in the food animals, the exposure of humans to this resistant organism through products derived from the animals, and then the consequence of this exposure to human health. The consequence is defined as the ranking of importance in GFI #152, Appendix A, where antibiotics medically important to human health are ranked as critically important, highly important, or important. This process has only been in place since approximately 2001, the date of the GFI #152 document; therefore, older antibiotics used in food animals have not undergone this process.

The second component of the microbial safety evaluation is described in GFI #159, also designated as Veterinary International Committee on Harmonization (VICH) GL36(R). The GFI #159 process involves the potential effect of daily intake of the antibiotic on two characteristics of the human intestinal microbiota; these are disruption of the colonization barrier and increase in populations of resistant bacteria in the human colon. An example of an antibiotic approved in the USA where the withdrawal time was based on the microbiological ADI rather than the toxicological ADI is tylvalosin, approved in 2012. The microbiological ADI was 47.7 μg/kg bodyweight per day, while the toxicological ADI was 190 μg/kg bodyweight per day (FDA, 2012b). Even with the lower ADI, analysis of residues indicated that no withdrawal time was necessary, and therefore no edible tissue tolerance was necessary.

Anticoccidials

Members of this class are presented in Table 52.2. Coccidia are a ubiquitous challenge in food animal species, with especially dire consequences if left unaddressed in chickens and turkeys. In fact, every anticoccidial in Table 52.2 is labeled for chickens. Coccidia may also be a significant health challenge for cattle, with two compounds labeled for feed. One of these is also labeled for water (amprolium), and one is also labeled for milk or milk replacer (decoquinate).

Other antimicrobial drugs that may also be used for anticoccidial effect in food animals include some of the sulfonamides (e.g., sulfaquinoxaline, sulfadimethoxine) and the polyether ionophores. In Europe, the ionophores would be included in the anticoccidial group also, rather than as an antimicrobial. This is an issue of semantics, as the ionophores are an antibiotic with anticoccidial properties. The anticoccidials are not thought to select for resistance to human pathogens, either directly or indirectly through coselection. Some food retailers have needlessly excluded the ionophores from use in their supply chains by demanding "antibiotic free" production systems without realizing that this included the ionophores in the United States. This is an example of where an attempt to achieve kudos for a marketing stance has resulted in the needless removal of a valuable animal health tool with no resulting discernible benefit to human health.

Antiinflammatories

Antiinflammatories are important in food animal veterinary practice and the four products primarily used in the United States are summarized in Table 52.2. Flunixin meglumine is the nonsteroidal antiinflammatory drug approved for food animal use in the United States for the control of pyrexia associated with bovine respiratory disease, acute bovine mastitis, and endotoxemia in cattle. In 2017, a new transdermal flunixin formulation was approved in the U.S. for treatment of pain associated with foot rot (infectious pododermatitis). It is also indicated for the control of inflammation in endotoxemia in cattle, and the control of pyrexia associated with swine respiratory disease. While it is commonly used as an adjunct therapy for respiratory disease in cattle, an analysis of available studies as well as the noninferiority study in the flunixin meglumine/florfenicol product label, indicate that benefits of therapy related to clinical outcome (clinical success) of bovine respiratory disease have not been demonstrated (Francoz et al., 2012; FDA, 2009).

Aspirin is also used in food animals and the containers for these drugs may carry indications for food animals. However, the labels on the containers for aspirin products used in food animals should not be confused with approval labels; no aspirin product for food animals in the United States is the subject of a new animal drug application approval (NADA) number granted by the FDA/CVM. Therefore, there is no tolerance for food animals concerning aspirin in the United States, and any amount detected would be violative as a residue. Some of the liquid aspirin products may be compounded from bulk product, which is specifically prohibited in the AMDUCA regulations. One use of aspirin is in liquid formulations through proportioners in water systems for swine, although a publication on serum pharmacokinetics of sodium salicylate after this route of application raises questions of bioavailability that are inconsistent with field reports of efficacy (Patterson et al., 2007). The use of oral boluses in cattle requires the need for frequent administration, at least twice daily, if adequate concentrations for pain relief are to be achieved (Apley, 2008).

Dexamethasone is a very commonly used steroid in food animal practice. Caution must be used in cattle due to their extreme sensitivity to immunosuppression, abortion, and adverse effects on semen quality in bulls. A dose of 0.04 mg/kg in cattle will inhibit neutrophil function for at least 24 hours after the last dose (Roth and Kaeberle, 1985). In pigs, it takes 2 mg/kg to create this effect. Dexamethasone has also been used as an adjunct

Table 52.2 Nonantibiotics labeled for food animal use in the United States by species and labeled routes of administration. These routes may be as part of a combination product, are approved specific to disease indications, and may also include only specific use classes and age restrictions within the species. This table contains label approvals as represented at *Animal Drugs@FDA*, the electronic version of the *Green Book* containing FDA CVM approvals.

Use classification	Class	Drug	Swine	Beef cattle, nonlactating dairy cattle	Lactating dairy cattle	Goats	Sheep	Chickens	Turkeys
Anticoccidials	Thiamine analog	Amprolium		F,W				F,W	F,W
	Pyridinols	Clopidol						F	F
	4-Hydroxyquinolone	Decoquinate		F,M		F	F	F	
	Triazine derivative	Diclazuril						F	F
	Nitrobenzamides	Dinitolmide (Zoalene)						F	F
	Alkaloid	Halofuginone						F	F
	–	Nicarbazin						F	
	Guanidine derivative	Robenidine						F	
Antiinflammatories	NSAIDS	Flunixin meglumine	I	I	I				
		Aspirin							
	Steroids	Dexamethasone		I,O,F	O				
		Isoflupredone acetate	I	I					
Production Enhancement	Beta-agonists	Ractopamine	F	F					F
		Zilpaterol	F						
	Injectable hormone	Bovine Somatotropin			I				
	Hormone implants	Estradiol		S					
		Progesterone		S					
		Testosterone		S					
		Trenbolone acetate		S					
		Zeranol		S					

Hormones – reproductive use		Progesterone		V				
		Altrenogest	F					
		Chorionic gonadotropin		I	I			
		Pituatary leuteinizing hormone	I	I			I	
		Follical stimulating hormone	I	I	I		I	
		Melengestrol acetate		F				
		Gonadorelin		I	I			
		Oxytocin	I	I	I		I	
		Cloprostenol		I	I			
		Fenprostaline	I	I				
		Dinoprost tromethamine						
Antiparasitics	Avermectins	Ivermectin	I,O,F	I,O,T			O	
		Eprinomectin		I,T	T			
		Doramectin	I	I,T				
	Milbemycins	Moxidectin		T,I			O	
	Benzimadazoles	Albendazole		O		O	O	
		Fenbendazole	F	O,F		O,F		
		Oxfendazole		O				
	Imidazothiazoles	Levamisole	W	I,O,W,T				
	Tetrahydropyrimidines	Morantel tartrate		F		F		
		Pyrantel tartrate	F					
	Aminoglycosides	Hygromycin B	F					F
	Organophosphates	Dichlorvos	F					
		Clorsulon		I,O				

F, feed; IMM, intramammary; I, injectable; IU, intrauterine; M, milk; O, oral; S, solid dose implant; T, topical; V, intravaginal; W, water.

therapy for thromboembolic meningoencephalitis and polioencephalomalacia in cattle. However, a review of the very limited clinical data combined with extrapolation of data from laboratory models and other species would indicate that the use of dexamethasone and flunixin meglumine in these applications may be equivocal at best, and may be likely to cause harm (Apley, 2015). Dexamethasone does not have a residue tolerance in the United States because the toxicity profile during the original drug approval did not merit a tolerance being created. Isoflupredone acetate has less adverse effect potential and more mineralocorticoid activity as compared to dexamethasone.

Extralabel use of other nonsteroidal antiinflammatory drugs, such as ketoprofen and meloxicam, have been utilized in food animals in the United States, with meloxicam gaining extensive popularity due to oral dosing potential, cost, and prolonged activity in cattle. Some other countries have multiple other options for these drugs. In the case of the use of both of these drugs, an exaggerated slaughter withdrawal time must be assigned by the attending veterinarian within a valid veterinary–client–patient relationship.

Production Enhancement Drugs

This general classification of drugs, summarized in Table 52.2, is unique to food animals. They are the subject of approved new animal drug applications and may not be used in an extralabel manner for production purposes. An example was where hormone implants were being used in calves intended for veal production even though this use class is not on the label. The FDA/CVM has made it quite clear that this extralabel use causes the animal to be adulterated.

Steroid implants for use in beef cattle are one of the most reliable efficiency increasing tools available to the beef industry; however, they have been the subject of controversy. Unsubstantiated reports have linked them to cases of early puberty in human females, but when one evaluates the added concentrations of hormones in beef from implanted cattle as compared to nonimplanted beef, and considers the amounts of estrogen and testosterone produced even in prepubescent humans, it becomes apparent that any contribution is minuscule. These standards are reflected in the approval requirements for endogenous sex steroid implants that contain estradiol, progesterone, or testosterone as described in Guidance for Industry number three (FDA, 2006). The FDA/CVM has concluded “that no additional physiological effect will occur in individuals chronically ingesting animal tissues that contain an increase of endogenous sex steroids from exogenous sources equal to 1% or less of the amount in micrograms produced by daily synthesis in the segment of the population with the lowest daily production.” For estradiol and progesterone, prepubertal boys provide the baseline benchmark at 6 and 150 μg per day, respectively. For testosterone, prepubertal girls provide the baseline at 32 μg per day. For synthetic sex steroids such as trenbolone, the FDA CVM recommends a series of laboratory animal tests.

Bovine somatotropin is approved in the United States with an indication of increasing production of marketable milk in healthy lactating dairy cows. As a protein, any amount of this drug in bovine milk will not have an effect on human physiology because this intact protein will not be absorbed in an active form from the gut. Any increase in insulin-like growth factor that occurs from ingestion of milk or other high protein and calorie source foods such as milk and eggs is most likely from the nutritive effect on the human body rather than any absorption of IGF from the food. Nevertheless, the use of bovine somatotropin in dairy cattle has become controversial in the United States and some dairy products are sold with the claim that this drug has not been used in their production.

The beta agonists act as repartitioning agents, with increased muscle and decreased fat produced from the nutritive inputs to the food animal. These drugs not only increase the efficiency of protein production in the animal, they also alter the proportions of different muscle groups in the body so that disproportionately more valuable muscle cuts are increased as compared to less valuable portions of the carcass. Zilpaterol has been the subject of controversy in the United States due to reported adverse health events related to cattle in the immediate preslaughter period when the drugs are administered and also at the slaughter plant. The actual cause of these noted events have been controversial, and at the time of this writing zilpaterol has been voluntarily removed from the market by the sponsor and evaluation of a new flexible dose label in the field is pending.

Ractopamine is approved in cattle, swine, and turkeys. Soon after approval, stress-related adverse events associated with ractopamine were noted in pigs being sent to slaughter. Education on the correct use of the drug as well as dose adjustment were used to alleviate these effects, and the drug remains in use in pigs at this time. Other beta agonists, such as clenbuterol, have been used for animal production in other countries, but this use is specifically prohibited in the United States.

The polyether ionophores are listed under antimicrobials in Table 52.1, but also are very commonly used performance-enhancing drugs in cattle. They are technically an antibiotic because they are a compound produced by one organism which inhibits the growth of another. Their mechanism of action is by inhibiting gram-positive bacteria in the rumen of cattle. This increases the proportion of bacteria that produce propionic acid, the most efficient of the volatile fatty acids for the ruminant animal to convert into glucose in the liver. The polyether ionophores also decrease methane production in the rumen, as well as acting as anticoccidials, as discussed in Section Anticoccidials.

Hormones for Reproductive Use

Reproductive management and therapy is a primary focus of food animal veterinarians and the products for reproductive use listed in Table 52.2 are important tools for this focus. Chapter 27 should be consulted for further details. All are injectable compounds except for altrenogest in swine (used for estrous synchronization) and melengestrol acetate (used for estrous suppression) in cattle. Another method of application is intravaginal devices that release progesterone. Estrous synchronization and super ovulation protocols for embryo collection in cattle are also routine uses of these products.

Controversy has arisen over extralabel use of compounds for reproductive purposes because this does not meet the requirement of “the health of an animal is threatened or suffering or death may result from failure to treat” as outlined in the AMDUCA regulations. Examples of these extralabel uses are lactation induction programs in dairy cattle and some estrous synchronization programs in cattle. The need in some reproductive protocols for an injectable estrogen in the absence of an approved compound has also led to the extralabel use of compounded estrogens. There has also been an interesting, but false, concept that injecting reproductive hormones closer to the reproductive tract will improve their efficacy. This has been used as an argument for injection in the hindquarters of the dairy cow rather than administering the drugs in the appropriate site in the neck.

Some of these hormones are used routinely as abortifacients in cattle. These include cloprostenol, fenprostaline, and dinoprost tromethamine. The use of all of these reproductive hormones require not only a clear understanding of the activity of the drug, but also the physiology within which they are applied.

Antiparasitics

The endectocides inhibit both internal and external parasites. The endectocides in Table 52.2 are macrocyclic lactones, consisting of the avermectins and milbemycins. The benzimidazoles are also referred to as the “white dewormers” due to the appearance of the formulation; these oral products act only on internal parasites. Chapters 39, 40, and 41 go into further details for this class of compounds. Some of the antiparasitics, as for the antimicrobials, are running into challenges with resistance in some animal and parasite species combinations. This issue of increasing importance prompted the FDA Center for Veterinary Medicine to host a stakeholder meeting and to publish a commentary on the meeting in 2014 (Kornele et al., 2014).

The primary species for resistance issues in food animals in North America and much of the world are sheep and goats, especially related to the *Haemonchus* species. Incorrect recommendations abound on the Internet for addressing this issue. Some sites suggest monthly deworming of small ruminants to counter the resistance problem, or that all of the products should be given orally (regardless of label route of indication) because that is “where the worms are”. Both of these concepts are incorrect. Others recommend combinations of products to address resistant parasites, which is a short-term solution. The correct approach to addressing resistant parasites in small ruminants is to only deworm when there is an infection that requires it, which means only in animals that are displaying signs of parasitism, to identify and eliminate the heaviest shedders, and to eliminate classic mismanagement such as overgrazing.

Anthelmintic resistance in nematodes of cattle has also been identified as a worldwide problem (Sutherland and Leathwick, 2011). While *Cooperia* species have classically been considered the nematode resistant to the ivermectins in cattle, there is also evidence that ivermectin resistance is being displayed in *Ostertagia ostertagia* and *Haemonchus* species (Gasbarre et al., 2009; Edmonds et al., 2010).

The obligation of the veterinarian to assure that anthelmintics are correctly selected and utilized has never been more important. As for the antibiotics, occasional new groups may arise but we likely have the majority of the antiparasitic compounds that will need to see us through the next decades. Applying principles of stewardship to assure their continued efficacy is critical.

Anesthetics and Analgesics

The biggest challenge with anesthetics and analgesics for food animals in the United States is that there is only one labeled option. Thiamylal sodium is labeled for injectable use in swine and cattle for anesthesia, but availability varies. The only model accepted by the United States FDA for establishing relief of pain in food animals is for foot rot in cattle. Other uses are considered extralabel for controlling pain in food animals.

For local analgesia, lidocaine is often the drug of choice. None of the lidocaine products with applications for food animals on the bottle are actually labeled for food animals. There is a lidocaine labeled for human use. The AMDUCA regulations allow for the extralabel use of this drug in food animals. Lidocaine should be used with caution in small ruminants as overzealous application may result in an overdose. Anesthesia is often accomplished in food animals through the extralabel use of xylazine, ketamine, and/or acepromazine. Butorphenol may be added to the regimen in some species for increased analgesia.

Nonsteroidal antiinflammatory drugs such as aspirin, flunixin meglumine, ketoprofen, and meloxicam are also used in an extralabel manner for pain in food animals and will require an extended slaughter withdrawal time. In order to avoid violative residues. Phenylbutazone displays an extended elimination half-time in cattle, which allows every other day dosing; however, this drug also displays prolonged slaughter residue characteristics and is prohibited from extralabel use in dairy cows 20 months of age or older. Injudicious use of this drug, especially in show animals, can lead to violative residues.

The need for labeled analgesics and anesthetics for food animals in the United States is critical.

Miscellaneous Food Animal Drugs

Other drugs with food animal labels include gastrointestinal therapeutics such as neostigmine for peristalsis initiation and poloxalene for reduction of bubbles in frothy bloat. There are diuretic drugs labeled for cattle, in oral forms of trichloromethiazide, furosemide, and multiple salts of thiazide. Furosemide is also labeled as an injectable for cattle.

Vitamin E, cupric glycinate (copper), and iron dextran or available as injectables. And, finally, corticotropin (ACTH) is available for beef and lactating dairy cattle.

Summary

While not comprehensive, this summary of drugs available for use in food animals highlights the unique complexities of extralabel use, residues and withdrawal times, consideration of human food safety, and the need to understand not only the animal physiology but also the relationship of the animal and disease to the production system and to the food chain.

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53

Pharmacology in Aquatic Animals

Ron A. Miller

Introduction

Antimicrobials have long been used to relieve pain and suffering and control infections in food-producing animals, including fish. The safe and prudent prescription of effective antimicrobials by veterinarians to treat aquatic animals has contributed immensely to the increased food production capacity of worldwide aquaculture. Use of antimicrobials in aquaculture is, however, not without risk. The American Veterinary Medical Association has published educational materials for veterinarians that describe prudent and judicious use guidelines for antimicrobials in aquaculture (AVMA, 2006). Antimicrobial-resistant bacteria, pathogenic to animals and humans, have been found in and near fish and shellfish farms where medicated feed has been administered (Damir et al., 2013; Miranda et al., 2013; Huys et al., 2001; Guardabassi et al., 2000; Sathiyamurthy et al., 1997; Husevag and Lunestad, 1995). In addition, fish have been implicated as potential reservoirs of zoonotic pathogens (Haenen et al., 2013), some of which may carry resistance genes, including extended-spectrum beta-lactamases (Sousa et al., 2011). Cabello (2006) suggests the unrestricted use of antimicrobials in aquaculture in any country has the potential to affect human and animal health on a global scale, and further suggests that this problem should be dealt with through unified local and global preventive strategies.

Three traditional antimicrobials are currently approved by the US Food and Drug Administration (FDA) for use in finfish and lobsters (Table 53.1). Continued use of the same antimicrobial(s) in the fish-rearing environment will likely increase the potential for emergence and selection of antimicrobial-resistant bacteria in the rearing facility and may diminish therapeutic effectiveness. A Canadian report stated there has been a significant reduction in antimicrobial usage in salmon farming since 2005 (Morrison and Saksida, 2013). Citing improvements in production, health management, and livestock selection, the authors noted few vaccines and limited chemotherapeutic options remain a concern.

In this chapter, many factors will be discussed that should be considered as aquatic animal veterinarians decide whether to prescribe the use of an antimicrobial in a finfish (population). The reader should consult earlier chapters in this text on antimicrobial drugs for further drug-specific discussions.

Legal Considerations when Selecting an Antimicrobial for Use in Aquatic Animals

Prior to making a decision to prescribe treatment for a fish or fish population, careful thought should be taken in selecting the most appropriate antimicrobial, dosage, and route of administration. Aquatic animal veterinarians can be faced with difficult decisions on which approved animal antimicrobial to use or whether to recommend an extralabel (ELU) use of an approved animal or human antimicrobial. In 1994, the Animal Medicinal Drug Use Clarification Act (AMDUCA) provided veterinarians in the US greater flexibility by allowing them to prescribe ELUs of certain approved animal and human drugs for animals under certain conditions. In 1996, the FDA published a regulation that established the conditions under which veterinarians may prescribe ELU of certain approved animal and human drugs for animals (Federal Register, 1996). Discussed here are four of the many key points outlined in the ELU regulations.

A Valid Veterinarian–Client–Patient Relationship

AMDUCA allows ELU only on the order of a licensed veterinarian in the context of a valid veterinarian–client–patient relationship (VCPR). A VCPR can only exist if the veterinarian: (i) assumes the responsibility for making medical judgments about the health of the animal(s) and the need for medical treatment (and the client has agreed to follow the veterinarian's instructions); (ii) has sufficient knowledge of the animal(s) to develop a diagnosis of the medical condition; and (iii) is readily available for follow-up in case adverse reactions or treatment

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Table 53.1 Antimicrobials approved for use in US aquaculture in poikilothermic food species. Source: US Food and Drug Administration.

Antimicrobial (manufacturer)	Species	Indication	Dosage regimen	Limitations/ Comments
Oxytetracycline dihydrate (Terramycin® 200 For Fish, Phibro Animal Health)	Pacific salmon	Mark skeletal tissue	250 mg/kg/day for 4 days	Salmon <30 g In feed as sole ration 7 day withdrawal time
	Salmonids	Control of ulcer disease (*Hemophilus piscium*), furunculosis (*Aeromonas salmonicida*), bacterial hemorrhagic septicemia (*A. liquefaciens*), and pseudomonas disease	2.5–3.75 g/100 lb/day for 10 days	In mixed ration No temperature restrictions 21-day withdrawal time
	Freshwater-reared salmonids	Control of mortality due to coldwater disease (*Flavobacterium psychrophilum*)	3.75 g/100 lb/day for 10 days	In mixed ration No temperature restrictions 21-day withdrawal time
	All freshwater-reared rainbow trout	Control of mortality due to columnaris disease (*F. columnare*)	3.75 g/100 lb/day for 10 days	In mixed ration No temperature restrictions 21-day withdrawal time
	Catfish	Control of bacterial hemorrhagic septicemia (*A. liquefaciens*) and pseudomonas disease	2.5–3.75 g/100 lb/day for 10 days	In mixed ration Water temperature not below 62°F (16.7°C) 21-day withdrawal time
	Lobster	Control of gaffkemia (*Aerococcus viridans*)	1 g/lb medicated feed for 5 days	In feed as sole ration 30-day withdrawal time
Oxytetracycline HCl (oxytetracycline HCl Soluble Powder-343®, Phoenix Scientific; Pennox 343®, PennField Oil, Terramycin-343® soluble powder, Pfizer; OxyMarine®, Alpharma; and Tetroxy® Aquatic, Cross Vetpharm Group)	Finfish fry and fingerlings	Mark skeletal tissues	200 to 700 mg oxytetracycline HCl (buffered)/L of water for 2 to 6 h	None
Sulfadimethoxine–ormetoprim (Romet-30®, Pharmaq AS)	Salmonids	Control of furunculosis (*A. salmonicida*)	50 mg/kg/day for 5 days	In feed 42-day withdrawal time
	Catfish	Control of enteric septicemia (*Edwardsiella ictaluri*)	50 mg/kg/day for 5 days	In feed 3-day withdrawal time
Florfenicol (Aquaflor®, Intervet)	Catfish	Control of mortality due to enteric septicemia (*E. ictaluri*)	10–15 mg/kg/day for 10 days	VFD antimicrobial 12-day withdrawal time
	Freshwater-reared finfish	Control of mortality due to columnaris disease (*F. columnare*)	10–15 mg/kg/day for 10 days	VFD antimicrobial 12-day withdrawal time
	Freshwater-reared salmonids	Control of mortality due to coldwater disease (*F. psychrophilum*) and furunculosis (*A. salmonicida*)	10–15 mg/kg/day for 10 consecutive days	VFD antimicrobial 15-day withdrawal time
	Freshwater-reared warmwater finfish	Control of mortality due to streptococcal septicemia (*S. iniae*)	10–15 mg/kg/day for 10 consecutive days	VFD antimicrobial 15-day withdrawal time
Sulfamerazine, Zoetis	Rainbow, brook, and brown trout	Control of furunculosis	10 g/100 lb/day for up to 14 days	In feed 21-day withdrawal time **Not currently marketed**

Approval applies only to the specific antimicrobial that is the subject of a new animal drug application (NADA) or abbreviated new animal drug application (ANADA); active ingredients from other sources (e.g., bulk antimicrobial from a chemical company or similar compounds made by companies other than those specified in the NADA) are not approved new animal antimicrobials.

Approval applies only to use of the antimicrobial for the indications and manner specified on the label.

VFD, Veterinary Feed Directive.

failure is encountered. Such a relationship can only exist when the veterinarian has recently seen and is personally acquainted with the care of the animal(s) by virtue of examination and/or by visits to the premises where the animal(s) are kept.

General Conditions for ELU

ELU is limited to situations where an animal's health is threatened or where the animal may suffer or die without treatment. Before a veterinarian can legally prescribe an approved animal or human drug one of these general conditions must also be met: (i) no animal drug is approved for the intended use; (ii) an animal drug is approved for the intended use, but the approved drug does not contain the active ingredient you require; (iii) an animal drug is approved for the intended use, but the approved drug is not in the required dosage form you require; (iv) an animal drug is approved for the intended use, but the approved drug is not in the required concentration; and (v) an animal drug is approved for the intended use, but the veterinarian has found, in the context of a valid VCPR, that the approved drug is clinically ineffective when used as labeled.

In the ornamental fish industry (nonfood-producing), veterinarians can prescribe an approved human drug for an ELU even if an approved animal drug is available if the other conditions of AMDUCA are met. Whether prescribing drugs for food-producing fish or ornamental fish, thorough recordkeeping is critical. Records of drugs used, condition treated, animal species treated, dosage administered, treatment duration, number of animals, and drug withdrawal parameters must be kept for 2 years or as required by federal or state law, whichever is greater. Thorough labeling of the drug dispensed on the veterinarian's order is critical, including the veterinarian's name, the name of the dispensing pharmacy, and directions for the end user similar to that described for recordkeeping.

Conditions for ELU in Food-Producing Animals

Prior to prescription of an approved animal or human drug for an ELU in a food-producing animal, the veterinarian must: (i) make a careful diagnosis and evaluation of the condition to be treated; (ii) have an appropriate medical rationale for using a specific drug; (iii) make sure the client maintains the identity of the treated animal(s) in the record; (iv) establish a substantially extended withdrawal time supported by scientific information, and, if such information is nonexistent, take appropriate measures to assure that the animal(s) and its food products will not enter the human food supply; and (v) ensure that no illegal residues, including antimicrobial residues, occur and that the client follows the established withdrawal time before marketing food products made from treated animals.

Antimicrobials Prohibited from ELU in Animals

Under the AMDUCA provisions, FDA has the right to prohibit ELU of certain drugs in animals. Currently, no approved antimicrobials are prohibited from ELU in companion animals (including aquarium or pet fish). The following drugs (both human and animal), families of drugs, and substances are prohibited for extralabel animal and human uses in food-producing animals:

- Chloramphenicol
- Clenbuterol
- Diethylstilbestrol (DES)
- Dimetridazole
- Ipronidazole and other nitroimidazoles
- Furazolidone and nitrofurazone
- Sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine);
- Fluoroquinolones
- Glycopeptides
- Phenylbutazone in female dairy cattle 20 months of age or older
- Cephalosporins (not including cephapirin) in cattle, swine, chickens, or turkeys:
  - For disease prevention purposes;
  - At unapproved doses, frequencies, durations, or routes of administration; or
  - If the drug is not approved for that species and production class.

The following drugs, or classes of drugs, which are approved for treating or preventing influenza A, are prohibited from ELU in chickens, turkeys, and ducks:

- Adamantanes
- Neuraminidase inhibitors.

Routes of Antimicrobial Administration in Fish

Table 53.2 shows antimicrobial dosages by various routes of administrations that have been reported in the literature (Reimschuessel et al., 2012). Many of these dosages are unapproved in many countries, and were used in many of these studies for the purposes of investigating the antimicrobial's pharmacokinetics (PK). Researchers and clinicians should consult their regulatory authorities for the approved antimicrobials and dosages in their respective countries.

Table 53.2 Antimicrobial dosages used in fish. Source: Reimschuessel 2012. Reproduced with permission of John Wiley & Sons.

Antimicrobial	Dosage	Interval	Route	Comments
Amikacin	5 mg/kg	q12h	IM	
	5 mg/kg	q72h × 3	IM	
Amoxicillin	25 mg/kg	q12h	PO	Rarely used due to few gram-positive pathogens
	40–80 mg/kg	q24h 10 days	PO	
Ampicillin	10 mg/kg	q24h	IM	Sharks
	10 mg/kg	q12h 7–10 days	PO	Sharks
	50–80 mg/kg	q24h 10 days	PO	
Aztreonam	100 mg/kg	q48h 7 days	IM/IP	Used by koi hobbyists
Azithromycin	30 mg/kg	q24h 14 days	PO	
Ceftazidime	22 mg/kg	q72–96h × 3–5	IM/IP	
Cefquinome	5–20 mg/kg	Single dose	IP	Dose used for determining PK
Chloramine-T	20 mg/l	1 h 4 days	Bath	
	2.5–20 mg/l	Flush (various)	Bath	Disinfectant control bacterial gill disease and parasites
	5–10 mg/l	1 h	Bath	
Ciprofloxacin	15 mg/kg	single dose	IM/IV	Dose used for determining PK[a]
Difloxacin	10 mg/kg	single dose	PO	Dose used for determining PK[a]
Dihydrostreptomycin	0.125 mg	single dose	IM/IV	Dose used for determining PK
	10 mg	single dose	PO	Dose used for determining PK
	10 mg/kg	q24h	IM	Sharks
Enrofloxacin	2.5–5.0 mg/l	5 h q24h 5–7 days	Bath	[a]
	30–50 mg/l	4–24h (various)	Bath	
	5–50 mg/kg	q24h × 5–10 days	PO	[a]
	2.5–10 mg/kg	Single dose	IM/IP/IV	Dose used for determining PK
Erythromycin	10–20 mg/kg	Single dose	IP	For BKD before spawning
	50–100 mg/kg	q24h 10–21 days	PO	
	2 mg/l	1 h	Bath	For BKD in eggs
Florfenicol	5–20 mg/kg	q24h 10 days	PO	Salmon
	10–15 mg/kg	q24h 10 days	PO	Dose approved by US FDA for select species
	40–50 mg/kg	q12–24h	PO, IM, IP	Red Pacu
	25–50 mg/kg	Single dose	IM	
Flumequine	10–500 mg/l	1–72 h	BATH	Increase dose in saltwater[a]
	5–50 mg/kg	q24h 5–10 days	PO	[a]
	30 mg/kg	Single dose	IM/IP	IP (and IM) dose levels remain at effective levels for 10 days[a]
	2–25 mg/kg	Single dose	IV	Dose used for determining PK
Fumagillin	30–60 mg/kg	Single dose	PO	Dose used for determining PK
	3–6 mg/kg	Single dose	IV	Dose used for determining PK
Furpyrinol	4–32 mg/L	5 h	Bath	
Gentamicin	3 mg/kg	q72h	IM	Very nephrotoxic to aglomerular fish Bath exposure does not achieve blood levels
	6 mg/kg	Each week	IM	Sharks
Kanamycin	50–100 mg/l	q72h 5 h × 3	BATH	Nephrotoxic some species Change water 50% between treatments
	50 mg/kg	q24h	PO	Nephrotoxic some species
	10–20 mg/kg	q24h	PO	Sharks
	20 mg/kg	q72h × 5	IP	Nephrotoxic some species
Lincomycin	40 mg/kg	q24h	PO	Japan
Marbofloxacin	10 mg/kg	q24h 1–3 days	PO	Dose used for determining PK
Miloxacin	60 mg/kg	q24h 6 days	PO	Japan
Nalidixic acid	13 mg/l	1–4 h	Bath	
	20 mg/kg	q24h	PO, IM, IV	Other doses used for PK studies
Neomycin	66 mg/l	q3 days × 3	Bath	Toxic to nitrifying bacteria in filter
	20 mg/kg	Single dose	PO	Sharks to prevent bloat, poorly absorbed from gut

(*continued*)

Table 53.2 (*Continued*)

Antimicrobial	Dosage	Interval	Route	Comments
Norfloxacin	30–50 mg/kg	q24h 5 days	PO	
Oxolinic acid	25 mg/l	0.25 h q12h × 3	Bath	
	0.15–1.5 mg/l	10 days	Bath	
	50–200 mg/l	1–72 h	Bath	
	10 mg/kg	q24h 10 days	PO	Freshwater species
	25–75 mg/kg	q24h 10 days	PO	Saltwater species
Oxytetracycline	10–50 mg/l	1 h	Bath	For superficial infections
	20–50 mg/l	5–24 h q24h 5–6 days	Bath	Change 50–75% water between treatments
	55–83 mg/kg	q24h 10 days	PO	Dose approved by US FDA for select species
	25–50 mg/kg	q24h 5–7 days	IM/IP	Produces high levels for several days when given IM
	3 mg/kg	q24h	IV	Red pacu
Piromidic acid	10 mg/kg	q24h 5–10 days	PO	Japan
Sarafloxacin	10–30 mg/kg	q24h 10 days	PO	[a]
Sulfadiazine–Trimethoprim	30–50 mg/kg	q24h 7–10 days	PO	
	125 mg/kg		IP	
Sulfadimethoxine–Ormetoprim	50 mg/kg	q24h 5 days	PO	Dose approved by US FDA for select species
Sulfamerazine	220 mg/kg	q24h 14 days	PO	
	200 mg/kg	q24h 10 days	PO	
Sulfamethoxazole–Trimethoprim	20 mg/l	5–12 h q24h 5–7 days	Bath	Change 50–75% water between treatments
	30 mg/kg	q24h 10–14 days	PO	
Tetracycline	80 mg/kg	Single dose	PO	
Thiamphenicol	20 mg/l	1 h	Bath	
	50 mg/kg	q24h 7–10 days	PO	Japan
Vetoquinol	25–40 mg/kg	Single dose	PO	
Virginiamycin	40 mg/kg	q24h 15 days	PO	

[a] Extralabel use of fluoroquinolones in food animals is prohibited by US FDA.
BKD, bacterial kidney disease.
Data obtained from many authors.

Injections

Injectable treatments using an antimicrobial are typically used in higher-valued fish (i.e., brood stock, some ornamental species, etc.) due to the labor and care involved in minimizing the potentially significant stress to the animal due to sedation and physical handling. This procedure can be a massive undertaking for commercial producers. Despite these challenges, advantages of administering antimicrobials by injection include assuring that all animals receive the desired dose. Oftentimes the route is intramuscular (IM), but can be intraperitoneal, intravascular, or intradorsal (caudal to the dorsal fin). IM injections are usually administered in epaxial muscles, above the lateral line and near the caudal fin. If an aminoglycoside (e.g., gentamicin) is to be used, care should be taken to inject cranial to the dorsal fin to avoid large doses entering the kidney.

Immersion Baths

Immersion or waterborne treatments are most useful when a large number of animals need to be treated, when limited stress to the animal(s) is important, and of course when it is likely that the pathogen will be exposed directly to the medicated solution (e.g., on surface of fish or gills). There are three classical ways of administering an antimicrobial via a waterborne treatment. *Static baths* (and dips) can be used by adding an antimicrobial solution directly to the holding system. *Flush treatments* are most common in flow-through systems where the entire dose is added and then the water flow is returned to slowly dilute the dose. *Constant flow treatments* are comprised of a constant dose injected into the water system from a stock solution.

The disadvantages of waterborne treatments include expense, waste, and potential environmental contamination. Biological filters may also be compromised due to killing the filter bacteria. A rapid rise in ammonia has been seen using therapeutic concentrations of erythromycin in a catfish recirculating system, but chloramphenicol, nifurpirinol, oxytetracycline, and sulfamerazine did not affect the filter function (Treves-Brown, 2000).

It is also important to consider the ability of an antimicrobial to be absorbed from the water. Lipophilic compounds under a molecular weight of 100 will more likely diffuse across the gills. Antimicrobials that are absorbed from the water include chloramines, dihydrostreptomycin, enrofloxacin, erythromycin, flumequine, furpyrinol, kanamycin, oxolinic acid, oxytetracycline, nifurpirinol, sulfadimethoxine, sulfadimidine, sulfamonomethoxine, sulfanilamide, sulfapyridine, sulfisomidine, and trimethoprim. Antimicrobials that are absorbed poorly or not at all include chloramphenicol and gentamicin (Treves-Brown, 2000; Reimschuessel et al., 2005).

Dipping Treatments

These are a shorter and more controlled method of administering bath treatments. The advantages of this type of treatment are reduced waste (thus reduced expense) and less environmental contamination. The disadvantage of this type of approach is the increased stress to the animals through handling. Therefore most dip treatments are done when fish are small or in pet/aquarium cases, but commercial aquaculture producers have used tarpaulins to contain the antimicrobial (Vavarigos, 2003), and more recently “well boats” to more effectively contain treatments (Burka et al., 2012). Localized topical treatment, often under light anesthesia, has been recommended for small external lesions in pet fish (Stoskopf, 1993; Noga, 1996).

Some studies have used either hyperosmotic infiltration (first high osmolarity, >1200 mOsm/l, then lower osmolarity containing the antimicrobial) or ultrasound treatments to try to improve permeability across the gills. Antimicrobial absorption and elimination can be affected by salinity under normal conditions, and the effects of hyperosmotic treatments have not been adequately studied. Certain antimicrobials that bind divalent cations (such as the tetracyclines) may have their bioavailability compromised by the addition of salts. Ultrasound treatments to enhance absorption may be feasible in a small aquarium setting but have not been studied extensively. Both hyperosmotic and ultrasound treatments are fairly stressful. They are mainly used for vaccination rather than antimicrobial treatment (Treves-Brown, 2000; Navot et al., 2004).

Oral

Oral treatments are the most feasible methods for large commercial aquaculture systems because they are the least stressful for the animals; however, sick fish may not eat, resulting in lessened antimicrobial exposure and compromised therapeutic effectiveness. This was shown in a study where the concentration of oxolinic acid was examined in Atlantic salmon (*Salmo salar*) treated during an outbreak of winter ulcer disease (*Moritella viscosa*). Oxolinic acid was detected in plasma and tissues of healthy fish, whereas levels were below the limit of detection in moribund and dead fish (Coyne et al., 2004a). The moribund and dead fish also had no food in their gastrointestinal (GI) tracts. These results indicate that the antimicrobial appears to help actively feeding healthy fish fight off the infection, whereas fish with clinical signs are anorexic and therefore not receiving the antimicrobial. Variable intake can also occur if fish vary in size. Larger fish will probably consume more of the medicated feed than their smaller, less vigorous counterparts. Palatability, especially of the sulfonamide products, can also be a problem (Poe and Wilson, 1989).

Absorption from the intestinal tract may vary from species to species. As mentioned, saltwater fish will drink and, therefore, antimicrobials may bind cations in the water in their intestinal tracts, affecting bioavailability. The formulation of the antimicrobial may either enhance or decrease absorption.

Various methods for administering oral medications include commercial medicated feed, custom surface-coated feeds, custom feeds (e.g., gelatin diets), medicated live feeds (e.g., *Artemia* grown in or fed antimicrobials), injecting food (e.g., small fish used for food) and tube feeding (Noga, 1996; Treves-Brown, 2000). Obviously, some of these techniques are appropriate only for pet/aquarium fish.

Clinical Approaches by Aquatic Animal Specialists

The importance of implementing responsible antimicrobial use guidelines in clinical practice has been advocated by prominent international organizations (AVMA, 2006; World Organization for Animal Health, 2013; FDA, 2014c). A major component to *responsible use* of an antimicrobial in veterinary medicine is the proper identification of the causative agent, employment of standardized antimicrobial susceptibility testing (AST) procedures (when available), and reporting of such results to a veterinarian. The reporting of susceptibility test results is derived from the interpretation of the laboratory susceptibility test data using clinical breakpoints to denote an isolate as being susceptible, intermediate, or resistant. It is ultimately the responsibility of the veterinarian to make a decision on the appropriate antimicrobial to use therapeutically.

In general, it is not uncommon to encounter the following decision process in an aquatic animal disease veterinary diagnostic laboratory: (i) admit sample to laboratory; (ii) identify causative agent based on gross pathology and/or phenotypic procedures; and (iii) prescribe antimicrobial therapy. However, ideally, the following process would be followed: (i) admit sample to laboratory; (ii) identify causative agent; (iii) conduct standardized AST (CLSI, 2006, 2014a); (iv) consult available interpretive criteria (CLSI, 2014a) or published susceptibility data distributions; and (v) prescribe antimicrobial treatment or alternatively consider improved animal husbandry practices.

Rigos and Smith (2013) published a review of the multitude of factors that should be considered when recommending antimicrobial therapy in an aquaculture setting. They outlined challenges including limited in vitro susceptibility testing procedures (CLSI, 2006, 2014a), limited interpretive criteria to inform the proper dosage to be administered to the animal such that it will be effective and minimize the emergence of antimicrobial resistance (CLSI, 2014b), and differences in the PK dependent upon temperature, salinity, and fish species.

Antimicrobial Susceptibility Testing and Interpretive Criteria

When veterinarians decide whether or not to treat fish with an antimicrobial, they should consider the anticipated PK of the antimicrobial agent in the target fish species under the given conditions (i.e., water temperature, salinity, hardness). They should also choose an antimicrobial that is effective against the disease. A well-controlled and standardized antimicrobial susceptibility test is the best means to obtain this information. A critical component of an AST method is its ability to accurately predict a clinical outcome following treatment and/or detect emerging resistance. In other words, does a likely susceptible in vitro AST result automatically mean therapy will be efficacious? Conversely, does a likely resistant in vitro test result mean therapy will not be efficacious? The purpose of an in vitro antimicrobial susceptibility test is not to mimic in vivo conditions, but rather to provide reproducible results that can be used to predict clinical outcome.

Three reproducible AST methods are currently available for use in veterinary medicine. *Agar and broth dilution susceptibility tests* result in a minimal inhibitory concentration (MIC) for a single bacterial isolate and provide the most clinical relevance, where the MIC may be directly related to an achievable tissue or plasma concentration in vivo. *Disk diffusion susceptibility tests* yield diameters of the zone of inhibition which provide no correlation with achievable concentrations in vivo, but are simple and very affordable tests to run. The *E-test* has also gained popularity as a simple nonstandardized diffusion-based susceptibility test resulting in an MIC shown to yield virtually identical results as the more traditional broth microdilution tests (Luber et al., 2003).

Prior to 2001, aquatic animal disease researchers commonly used AST methods and clinical breakpoint values (susceptible, intermediate, and resistant) developed in their own laboratories. Different methods and breakpoint values prevented accurate interlaboratory comparisons and correlation to clinical cases. Currently, there are two Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards, NCCLS) guidelines for disk diffusion and broth microdilution AST of bacteria isolated from aquatic animals (CLSI, 2007, 2014a). These guidelines have provided standardized methods and quality control procedures that have allowed testing of nonfastidious aquaculture pathogens (termed Group 1 organisms) on unsupplemented Mueller–Hinton media, and *Flavobacterium columnare* and *F. psychrophilum* (Group 3 organisms) on diluted Mueller–Hinton media, in such a way that is reproducible and more reliable (Table 53.3). These documents also describe recommended nonstandardized methods for some of the more fastidious pathogens (e.g., halophilic *Vibrio* spp., streptococci, etc.) (Table 53.4).

How does one decide if an isolate is susceptible, resistant, or intermediate based on a single AST result? The data obtained from the test – MIC or zone diameter – must be interpreted based on potential clinical effect. These interpretive criteria or breakpoints are determined by a number of considerations. MIC clinical breakpoint values are determined from three main sources of information (CLSI, 2007a):

1) PK and/or pharmacodynamic (PD) studies of the antimicrobial in the target animal species used to determine the likelihood of achieving a concentration of the antimicrobial at the target site (resulting in a PK/PD cutoff value, μg/ml).
2) Historical clinical outcomes are correlated with MICs of clinical isolates evaluated (resulting in a *clinical cutoff* value, μg/ml).
3) The antimicrobial MIC distribution for the pathogen is examined to develop epidemiologic cutoffs (resulting in an epidemiological cutoff, ECOFF, μg/ml).

Zone diameters from disk diffusion tests can also be used to interpret an isolate's level of susceptibility. Zone diameter clinical breakpoints are determined primarily from large AST data distributions where each isolate's diameter of zone of inhibition is plotted on the x-axis and its MIC is plotted on the y-axis (Miller and Reimschuessel, 2006; Uhland and Higgins, 2006). Regression analysis has often been used to suggest appropriate zone diameter breakpoint values, but is dependent upon a fairly even distribution of organisms at each MIC tested, particularly in the range of the intermediate MIC ± two to three twofold dilutions (Fuchs et al., 2002). With many of

Table 53.3 Standard methods for broth dilution susceptibility testing of aquatic bacterial pathogens. Source: Reprinted with permission by the Clinical and Laboratory Standards Institute (CLSI) from M42/M49-S1 (Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Second Informational Supplement, VET03/VET04-A). www.clsi.org.

Organism	Medium	Incubation
Group 1: Nonfastidious bacteria		
Enterobacteriaceae	CAMHB	22°C (24–28 hours and/or 44–48 hours) or
Vibrionaceae		28°C (24–28 hours)
Aeromonas salmonicida (typical and atypical strains)		
Aeromonas hydrophila and other mesophilic Aeromonads		
Pseudomonas spp.		
Plesiomonas shigelloides		
Group 3: Gliding bacteria[a]		
Flavobacterium columnare	Diluted CAMHB (4 g/l)	28°C (44–48 hours)
Flavobacterium psychrophilum	Diluted CAMHB (4 g/l)	18°C (92–96 hours)

CAMHB, cation-adjusted Mueller-Hinton broth; NaCL, sodium chloride.

[a] Some modification may be necessary for testing *Flavobacterium branchiophilum,* which may include cations, horse, or fetal calf serum, or NaCl.

Table 53.4 Potential modifications for broth dilution susceptibility testing of aquatic bacterial pathogens. Source: Reprinted with permission by the Clinical and Laboratory Standards Institute (CLSI) from M42/M49-S1 (Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Second Informational Supplement, VET03/VET04-A2). www.clsi.org

Organism	Medium	Incubation
Group 2: Strictly halophilic Vibrionaceae	CAMHB + NaCl (1%)	22°C (24–28 hours and/or 44–48 hours) or 28°C (24–28 hours and/or 44–48 hours)
Group 4: Streptococci		
Lactococcus spp.	CAMHB + LHB (2.5% to 5% v/v)	22°C (44–48 hours + CO_2 if necessary for growth)
Vagococcus salmoninarum		
Streptococcus spp.	CAMHB + LHB (2.5% to 5% v/v)	28°C (24–28 hours and/or 44–48 hours + CO_2 if necessary for growth)
Carnobacterium maltaromaticum		
Other streptococci		
Group 5: Other fastidious bacteria		
Atypical *Aeromonas salmonicida*	CAMHB	15°C (44–48 hours)
Aliivibrio salmonicida (formerly *Vibrio salmonicida*) and *Moritella viscosa*	Unknown	4°C or 15°C (6 days)
Tenacibaculum maritimum	Diluted MHB (1:7) + inorganic ion supplementation	25°C (44–48 hours)
Francisella spp.	Modified CAMHB with 2% defined growth supplement (Soto et al., 2010) and 0.1% glucose	28°C (24–48 hours)
Renibacterium salmoninarum	KDM-2	15°C (96 hours with agitation)
Mycobacterium spp.	(CLSI, 2011)	(CLSI, 2011)
Nocardia spp.		
Piscirickettsia salmonis	AUSTRAL-SRS (Yañez et al., 2012)	18°C (92–96 hours and/or 116–120 hours)

CAMHB, cation-adjusted Mueller–Hinton broth; KDM-2, kidney disease medium-2; LHB, lysed horse blood, MHB, Mueller–Hinton broth; NaCL, sodium chloride.

the newer antimicrobials (e.g., florfenicol), encountering resistant organisms is uncommon, and the MIC distribution is heavily weighted toward very susceptible MICs, resulting in an unreliable regression line. Researchers have developed statistically based algorithms to calculate MIC ECOFFs (Turnidge et al., 2006; Kronvall, 2010), which can then be used for surveillance and/or setting clinical breakpoints. The *Aeromonas salmonicida*

MIC and zone diameter ECOFFs for gentamicin, erythromycin, florfenicol, sulfadimethoxine–ormetoprim, sulfamethoxazole–trimethoprim, oxytetracycline, and oxolinic acid were established by the CLSI solely based on a visual inspection of the distribution data (Miller and Reimschuessel, 2006; CLSI, 2014b) (Table 53.5). It is anticipated that the *A. salmonicida* ECOFFs and clinical breakpoints will be revisited by the CLSI's Aquaculture Working Group once an internationally harmonized, statistically based method is endorsed by the CLSI.

By definition, a clinical breakpoint is the classification of projected clinical outcome of patient treatment based on the causative microorganism's in vitro response to an antimicrobial relative to the exposure to that agent that is attainable using the labeled dose regimen for the target animal species for that type of infection and infecting organism (CLSI, 2007a). Thus, the bacterial species – and target animal species – specificity of these values should provide the clinician a level of confidence in these values. It is important to note that interpretive criteria apply only if the laboratory has conducted AST according to specific standardized methods.

Currently, the only universally accepted clinical breakpoint values available for any fish pathogen are those provided in CLSI's VET03/VET04 Supplement (CLSI, 2014b). Provided in that supplement are oxytetracycline and oxolinic acid clinical breakpoints for *Aeromonas salmonicida* (Table 53.6). Clearly, there is a need for additional interpretive criteria that will be useful to aquatic animal health specialists. Future metaanalyses of data taken from the massive PK database published by Reimschuessel et al. (2005), along with additional in vitro MIC and zone diameter distributions can be used to help determine clinical breakpoints in aquaculture. Additional PK/PD investigations may also be needed to determine PK/PD index targets (i.e., time above MIC, AUC/MIC, C_{max}/MIC) most appropriate and applicable to fish.

Timing of Treatment

In aquatic animal medicine, veterinarians are placed in a unique position where vastly different effectiveness rates can be expected depending upon the stage of the disease in the target animal/population. Medication of farm-raised fish in situ is typically metaphylactic. Timing of treatment is critical to preventing de novo infections of healthy members of the treated population. Sicker animals will consume medicated feed at a lower rate than healthier animals. Coyne et al. (2004a) showed 85% of moribund farm-raised rainbow trout that were offered oxolinic acid-medicated feed had no detectable plasma antimicrobial levels, whereas 95% of the healthy fish had detectable levels.

Salinity Effects

The salinity of the surrounding water may induce an effect on the elimination rate of antimicrobials in fish (Choo, 1997). Considerably lower oxytetracycline concentrations were measured in the muscle of treated fish held in seawater. The reduced absorption of oxytetracycline in marine environments is likely due to the complexes the molecule forms with cations found in the water and in the feed. Increased rate of glomerular filtration in individuals in the marine environment may also contribute to the lower absorption. Considerations by practitioners of the salinity and hardness of the rearing systems should always be factored when designing treatment schedules.

Temperature Effects

Temperature has been shown in many PK studies in fish to be an important factor that can affect the PK of many antimicrobials used in aquaculture. This temperature effect may be related to an increased gastric emptying rate, resulting in a faster kinetic profile (absorption, elimination) and lower tissue concentrations of some antimicrobials (oxytetracycline) with increasing temperature.

Antimicrobials Used in Aquaculture

Oxytetracycline

Oxytetracycline is a broad-spectrum antimicrobial widely used in aquatic animal medicine, partly due to its lower order of toxicity, ability to readily distribute throughout the body and into tissues (Chambers, 2001), and general attractiveness from a cost perspective. Oxytetracycline and other tetracyclines are primarily bacteriostatic, and inhibit bacterial protein synthesis by preventing the association of aminoacyl-tRNA with the bacterial ribosome.

Terramycin® 200 for Fish (oxytetracycline dihydrate) Phibro Animal Health is approved by the FDA as an oral antimicrobial to treat specific diseases in salmonids, catfish, and lobster (Table 53.1; FDA, 2014a). The FDA-approved doses or dose ranges are consistent with those approved and/or used in other countries. However, curiously, very few studies in finfish have investigated the PK of oxytetracycline during or immediately after a multiday medicated feed dosing regimen using the FDA-approved dose (2.5–3.75 g/100 lb BW/day or 55–83 mg/kg BW/day). Chen et al. (2004) and Coyne et al. (2004b) both offered ad libitum a dose of 3.75 g/100 lb/day for 10 days and captured oxytetracycline systemic concentrations solely during the depletion phase of the concentration–time profile.

Table 53.5 Zone diameter and minimal inhibitory concentration epidemiological cutoff values for *Aeromonas salmonicida*. Source: Reprinted with permission by the Clinical and Laboratory Standards Institute (CLSI) from M42/M49-S1 (Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Second Informational Supplement, VET03/VET04-A2). www.clsi.org

Testing Conditions

Medium: Mueller–Hinton agar
Inoculum: Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation: 22°C ± 2°C; ambient air; 44 to 48 hours

QC Recommendations

(see Tables 6 and 7 in CLSI document VET03 (CLSI, 2006) for acceptable QC ranges)
Escherichia coli ATCC® 25922[a]
Aeromonas salmonicida ATCC® 33658

General Comments

1. These ECVs are applicable only to isolates of *A. salmonicida* tested under quality controlled conditions, as described in CLSI document VET03 (CLSI, 2006) and VET04-A2 (CLSI, 2014a). Before interpreting results for test strains, ensure that QC test results are within the ranges specified in this supplement.
2. ECVs presented here were established **solely based on** visual inspection of MIC and/or zone diameter data associated with an epidemiological database derived from geographically diverse diagnostic laboratory surveys. **However, it is expected that recently developed statistical analyses will be applied to all susceptibility data distributions to be used for establishing ECVs.** ECVs can be used as a measure of the emergence of strains with reduced susceptibility to a given agent. They are not clinical breakpoints, and, thus, proven clinical relevance has not yet been identified or approved by CLSI or any regulatory agency.
3. The isolates used to establish these ECVs were not from fish that were part of a clinical field trial. These ECVs are to be used in the establishment of interpretive criteria, as described in Appendix C of CLSI document VET02.
4. A "wild-type" (WT) cutoff category implies isolates are susceptible to the antimicrobial (no resistance mechanisms). A "non-wild-type" (NWT) cutoff category implies the isolates possess acquired and/or mutational resistance mechanisms.

NOTE: Information in boldface type is considered **new or modified since initial edition of the VET03/VET04-S.**

Antimicrobial Agent	Disk Content	Zone Diameter Cutoff (mm)		MIC Cutoff (μg/ml)		Comments
		WT	NWT	WT	NWT	
AMINOGLYCOSIDES						
Gentamicin	10 μg	≥ 18	≤ 17	–	–	Established based on **visual inspection of** a zone diameter distribution of 106 *A. salmonicida* isolates (Smith et al., 2007)
MACROLIDES						
Erythromycin	15 μg	≥ 14	≤ 13	–	–	Established based on **visual inspection of** a zone diameter distribution of 106 *A. salmonicida* isolates (Smith et al., 2007)
PHENICOLS						
Florfenicol	30 μg	≥ 27	≤ 26	≤ 4	≥ 8	Established based on **visual inspection of** zone diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007)
FOLATE PATHWAY INHIBITORS						
Ormetoprim–sulfadimethoxine	1.25/23.75 μg	≥ 20	≤ 19	≤ 0.5/9.5	≥ 1/19	Established based on **visual inspection of** zone diameter and MIC distributions of 217 *A. salmonicida* isolates (Miller and Reimschuessel, 2006)
Trimethoprim–sulfamethoxazole	1.25/23.75 μg	≥ 20	≤ 19	–	–	Established based on **visual inspection of** zone diameter distribution of 106 *A. salmonicida* isolates (Douglas et al., 2007)
TETRACYCLINES						
Oxytetracycline	**30 μg**	**≥ 28**	**≤ 27**	**≤ 1**	**≥ 2**	**Class representative for tetracyclines. Established based on visual inspection of zone diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007)**
QUINOLONES						
Oxolinic acid	**2 μg**	**≥ 30**	**≤ 29**	**≤ 0.12**	**≥ 0.25**	**Established based on visual inspection of zone diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007)**

ATCC, American Type Culture Collection; ECV, epidemiological cutoff values; MIC, minimal inhibitory concentration; NWT, non-wild-type; QC, quality control; WT, wild-type.

[a] ATCC is a registered trademark of the American Type Culture Collection.

Table 53.6 Zone diameter and minimal inhibitory concentration breakpoints for *Aeromonas salmonicida*. Source: Reprinted with permission by the Clinical and Laboratory Standards Institute (CLSI) from M42/M49-S1 (Performance Standards for Antimicrobial Susceptibility Testing of Bacteria Isolated from Aquatic Animals; Second Informational Supplement, VET03/VET04-A2). www.clsi.org.

Testing Conditions

Medium:	Mueller–Hinton agar
Inoculum:	Growth method or direct colony suspension, equivalent to a 0.5 McFarland standard
Incubation:	22°C ± 2°C; ambient air; 44 to 48 hours

QC Recommendations

(see Tables 6 and 7 in CLSI document VET03 (CLSI, 2006) for acceptable QC ranges)
Escherichia coli ATCC®* 25922[a]
Aeromonas salmonicida ATCC® 33658

General Comments

1. These breakpoints are applicable only to isolates of *A. salmonicida* tested under quality controlled conditions, as described in this supplement and in CLSI document VET03 (CLSI, 2006) and VET04-A2 (CLSI, 2014a). Before interpreting results for clinical strains or test strains, ensure that QC test results are within the ranges specified in this supplement.
2. **The papers cited as providing relevant clinical correlation data to support these clinical breakpoints all report work performed before the development of the standard susceptibility test protocols in CLSI document VET03 (CLSI, 2006) and in VET04 (CLSI, 2014a). Consequently, these breakpoints should be updated as data are generated. Additionally, the oxytetracycline and oxolinic acid ECVs used in support of these breakpoints (see Table 5 were established solely based on visual inspection. However it is expected that recently developed statistical analyses will be applied to all susceptibility data distributions to be used for establishing ECVs.**
3. **These breakpoints were established from studies of the treatment of Atlantic salmon in fresh water and seawater. Caution should be taken in extrapolation of these breakpoints to treatments other than those under which they were established.**
4. Antimicrobial susceptibility test breakpoints or interpretive categories provide a classification of projected clinical outcome of animal treatment based on the causative microorganism's *in vitro* response to an antimicrobial agent relative to the exposure to that agent, which is attainable using the normal dose regimen for the target animal species for that type of infection and infecting organism. A "susceptible" (S) breakpoint category implies that an infection due to the isolate may be successfully treated with the normal dosage regimen of an antimicrobial agent recommended for that type of infection and infecting species, unless otherwise indicated. An "intermediate" (I) category implies that an infection due to the isolate may be successfully treated in body sites where the drugs are physiologically concentrated; it also indicates a "buffer zone" that should prevent small, uncontrolled, technical factors from causing major discrepancies in interpretations. A "resistant" (R) category implies isolates are not inhibited by the usually achievable concentrations of the agent with normal dosage regimens and/or fall in the range where specific microbial resistance mechanisms are likely, and clinical efficacy has not been reliable in treatment studies.
5. **The normal dose regimen for oxytetracycline was taken to be approximately 75 mg/kg/day for 10 days. For oxolinic acid, it was taken to be approximately 10 mg/kg/day for 10 days.**

NOTE: Information in boldface type is considered **new or modified since the initial edition of the VET03/VET04-S.**

Antimicrobial Agent	Disk Content	Zone Diameter Breakpoint (mm)			MIC Breakpoint (µg/ml)			Comments
		S	I	R	S	I	R	
Tetracyclines								
Oxytetracycline	30 µg	≥ 28	22–27	≤ 21	≤ 1	2–4	≥ 8	**Class representative for tetracyclines.** Established based on: 1. **Visual inspection of** zone diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007) 2. Clinical correlations (Coyne et al., 2004b) – **Atlantic salmon held at 13°C in freshwater commercial conditions and dosed *ad libitum* 75 mg/kg body weight for 10 consecutive days (Pursell, 1998) – Atlantic salmon held at 14°C in freshwater laboratory conditions and dosed *ad libitum* 75 mg/kg body weight for 10 days.** 3. **PK data (Chen et al., 2004) – Various nonsalmonid species held at 15 to 30°C in fresh/saltwater laboratory conditions and dosed *ad libitum* 82.8 mg/kg body weight for 10 days (Miller et al., 2012) – Rainbow trout held at 12°C in freshwater laboratory conditions and dosed by gavage 74 mg/kg body weight for 10 days.**

(*continued*)

Table 53.6 *(Continued)*

Antimicrobial Agent	Disk Content	Zone Diameter Breakpoint (mm)			MIC Breakpoint (µg/ml)			Comments
		S	I	R	S	I	R	
Quinolones								
Oxolinic acid	2 µg	≥ 30	25–29	≤ 24	≤ 0.12	0.25–0.5	≥ 1	Established based on: 1. **Visual inspection of z**one diameter and MIC distributions of 323 *A. salmonicida* isolates (Miller and Reimschuessel, 2006; Smith et al., 2007) 2. Clinical correlations (O'Grady et al., 1987) – **Atlantic salmon held at 10°C in freshwater commercial conditions and dosed *ad libitum* 10 mg/kg body weight for 10 days (Hastings and McKay, 1987) – Atlantic salmon held at 14 to 16°C in saltwater commercial conditions and dosed *ad libitum* 20 mg/kg body weight for 2 days, then 10 mg/kg body weight for 4 days (Smith and O'Grady, 2006) – Atlantic salmon held at 10°C in freshwater experimental conditions, experimentally challenged, and dosed *ad libitum* 10 mg/kg body weight for 10 days.** 3. **PK data (Samuelsen et al., 2003b) – Cod held at 8°C in saltwater laboratory conditions and dosed intravenously once with 12.5 mg/kg body weight (Björklund and Bylund, 1991) – Rainbow trout held at 16°C in freshwater laboratory conditions and dosed intravenously once with 10 mg/kg body weight.**

ATCC, American Type Culture Collection; ECV, epidemiological cutoff value; I, intermediate; MIC, minimal inhibitory concentration; PK, pharmacokinetic; QC, quality control; R, resistant; S, susceptible.

[a] ATCC is a registered trademark of the American Type Culture Collection.

Chen et al. reported maximum serum concentrations 0.04 days after the 10-day regimen of 1.25–5.18 µg/ml in nonsalmonid species. Coyne et al. reported mean plasma concentrations 1 day after the 10-day regimen of 0.25 and 0.21 µg/ml in Atlantic salmon (*Salmo salar*) reared in farm and laboratory conditions, respectively.

Miller et al. (2012) investigated the oxytetracycline pharmacokinetic profile in laboratory-held rainbow trout (*Oncorhynchus mykiss*) fed 74.7 mg/kg/day via oral gavage and reported a mean maximum serum concentration of approximately 1 µg/ml (Figure 53.1).

In June 2006, the FDA approved a supplemental new animal drug application (NADA) which changed the Terramycin® 200 For fish oxytetracycline formulation from the monoalkyl (C8-C18) trimethylammonium or q-salt, to the dihydrate salt (FDA, 2014b). A pharmacokinetics study in shrimp compared oxytetracycline-medicated feeds prepared with the q-salt form and the dihydrate form. This study showed the oxytetracycline q-salt form remained in the environment longer than the oxytetracycline dihydrate form (Reed et al., 2006). The authors suggested the q-salt form is probably more detrimental to the environment and fauna than the dihydrate form.

Sulfonamides and Potentiated Sulfonamides

Sulfadimethoxine–ormetoprim (5 : 1 ratio) is approved by the FDA as an oral antimicrobial to treat furunculosis in salmonids and enteric septicemia in catfish (Table 53.1).

Individually, sulfadimethoxine and ormetoprim are active against a wide array of pathogenic microorganisms. However, when administered in combination, they work synergistically and may lower the incidence of antimicrobial resistance (Bullock et al., 1974). Sulfonamides like sulfadimethoxine are p-aminobenzoic acid (PABA) analogs, which competitively inhibit the incorporation of PABA into folic acid. This prevents the synthesis of folic acid and subsequent bacterial growth. Ormetoprim serves as a potentiator of the antifolate effect of sulfonamides by competitive inhibition of dihydrofolate reductase. Together they offer broad-spectrum antimicrobial activity, which is reported to be effective for treating infections in fish caused by *Yersinia ruckeri* (enteric redmouth disease) (Bullock and Snieszko, 1979), *Aeromonas salmonicida* (furunculosis) (Bullock et al., 1974), and *Edwardsiella ictaluri* (enteric septicemia in catfish) (Plumb et al., 1987).

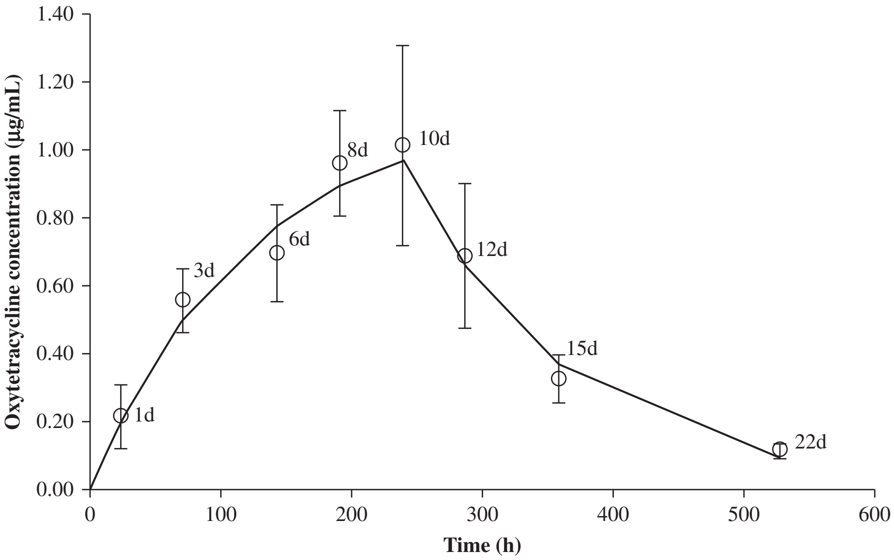

Figure 53.1 Serum oxytetracycline concentrations (mean ± SD; open circles) in rainbow trout after oral administration of medicated feed for 10 consecutive days. The solid line represents the population predicted mean value at each sampling time using a regimen of 75 mg oxytetracycline/kg daily for 10 consecutive days. Source: Miller et al., 2012. Reproduced with permission of Taylor & Francis.

In addition to sulfadimethoxine-ormetoprim, other sulfonamides and potentiated sulfonamides are approved for use in some countries. Sulfamonomethoxine, sulfisozole, and sulfadimethoxine are all approved for use in Japanese aquaculture. Sulfamerazine is approved for use in some countries in Europe. Sulfadiazine–trimethoprim (Tribrissen®) and sulfamonomethoxine–ormetoprim are used in Europe, Canada, and Japan. Romet-30® and these other potentiated sulfonamides have shown potent activity against several fish pathogenic species including *A. salmonicida*, *V. anguillarum*, and *Aliivibrio salmonicida* (formerly *V. salmonicida*) (Samuelsen et al., 1997; Hoie et al., 1992).

Sulfamerazine is approved by the FDA as an oral antimicrobial to treat furunculosis in trout, but is no longer marketed.

Florfenicol

Florfenicol is a broad-spectrum, primarily bacteriostatic antimicrobial with a range of activity similar to that of chloramphenicol. Florfenicol, however, does not carry the risk of inducing human aplastic anemia that is associated with chloramphenicol. The mode of action of florfenicol is by binding to the 50S ribosomal subunit and inhibition of the transpeptidyl-transferase step in protein synthesis, effective against gram-negative and gram-positive bacteria.

Aquaflor® (florfenicol) is approved by the FDA as an oral antimicrobial to control several diseases in catfish and freshwater-reared finfish (Table 53.1).

Florfenicol is also commonly used in Japan, Canada, and Europe to treat furunculosis, columnaris, and other diseases. Cyanphenicol and thiamphenicol are members of the phenicol family and are approved for use in Japan. Despite being banned from use in aquaculture-producing countries in Asia and Southeast Asia, chloramphenicol has been detected in farmed shrimp imported into the European Union. Chloramphenicol is on the FDA's list of prohibited substances for ELU in all food-producing animals due to its link to human aplastic anemia, intestinal problems, and neurological reactions (US FDA, 2017). The European Union (EU) established a minimum required performance limit (MRPL) for this compound of 0.3 µg/kg in tissues from food animals (EU, 2007).

Quinolones

Oxolinic acid is a first-generation synthetic quinolone antimicrobial that possesses potent activity against many bacterial fish pathogens, especially gram-negative organisms. It is accepted for use in aquaculture in Japan and some countries in Europe, being widely used in Norwegian aquaculture (Grave et al., 1999) to treat numerous diseases including furunculosis. Oxolinic acid is administered orally via medicated feed with a recommended dose for finfish of 12 mg/kg BW/day for up to 7 days (EMA, 2005). Samuelsen and Bergh (2004) showed significantly decreased mortality in fish offered oxolinic acid-medicated feed versus the controls after a *Vibrio anguillarum* immersion challenge.

Flumequine similar to oxolinic acid, is a first-generation quinolone active against mainly gram-negative bacteria, and approved for use in Japan and some European countries. Flumequine inhibits DNA-gyrase (Drlica and Zhao, 1997). Vik-Mo et al. (2005) showed efficacy in laboratory trials against an experimental infection caused by *Listonella anguillarum*. Flumequine is also used in shrimp farming to combat vibriosis; however, a recommended dosage has not been determined (FAO/WHO, 2006).

Other quinolone antimicrobials like nalidixic acid and piromidic acid, are approved in Japan, but very little is known regarding the recommended dose, pharmacokinetics, withdrawal times, or extent of their use (Jarboe et al., 1993; Uno et al., 1992; Katae et al., 1979a, 1979b).

Beta-lactams

Amoxicillin is likely the most commonly used beta-lactam antimicrobial in aquaculture worldwide. It is approved for use in Japan and some European countries (Schnick, 2001) to treat furunculosis and other bacterial diseases. The recommended dose is 40–80 mg/kg BW/day for 10 consecutive days (Roberts and Shepherd, 1997). Amoxicillin inhibits cell wall synthesis by preventing peptidoglycan cross-linkage, thus it is primarily active against gram-positive bacteria. In mammalian medicine, amoxicillin is administered with clavulanic acid to decrease its susceptibility to degradation by beta-lactam producing bacteria and to increase the spectrum of action against gram-negative bacteria. However, this has not yet been reported in the aquaculture literature. Amoxicillin is the antimicrobial of choice within the class because it is better absorbed following oral administration than other beta-lactam antimicrobials (i.e., ampicillin) (Prescott, 2006).

Pharmacokinetics of Antimicrobials Commonly Used in Aquaculture in the USA

Oxytetracycline

For many years researchers have investigated the absorption, distribution, metabolism, and elimination profiles of oxytetracycline in various fish and shellfish species (Rigos et al., 2004, 2011; Zhang and Li, 2007; Uno et al., 2006; Reed et al., 2006; Chen et al., 2004; Wang et al., 2004; Coyne et al., 2004b; Bernardy et al., 2003; Haug and Hals, 2000; Abedini et al., 1998; Doi et al., 1998; Du et al., 1997).

Absorption

Tetracyclines are known for their poor absorption from the GI tract, with oxytetracycline categorized as intermediate, meaning when the stomach is empty 60–80% of an oral dose is absorbed. Oxytetracycline absorption in mammals is decreased in the presence of dairy products, calcium, magnesium, and iron or zinc salts in the GI tract. Divalent and trivalent cations bind oxytetracycline decreasing its antimicrobial activity (Lunestad and Samuelsen, 2001). This can be particularly important in aquaculture when oxytetracycline is administered to fish in seawater where cation levels are high (Barnes et al., 1995). Unavoidable contact with seawater occurs when oxytetracycline surface-coated feed pellets are offered. Contact can also occur in the upper GI tract of marine teleost fishes that characteristically drink seawater continuously to compensate for water loss. Encapsulation of oxytetracycline in the feed pellet may avoid direct antimicrobial–cation interaction; however, for absorption to take place the antimicrobial must be in the liquid form, thus mixing with cation-rich fluid in the gut is unavoidable.

Few researchers have reported absorption half-lives ($T_{1/2\alpha}$) of oxytetracycline in fish following nonintravenous routes of administration. Wang et al. (2001) reported a $T_{1/2\alpha}$ of 2.3 hours in orally administered black seabream (*Sparus macrocephalus*).

Bjorklund and Bylund (1990) showed absorption of oxytetracycline in freshwater fish was faster at higher temperatures. At 16°C, the maximum plasma concentration (C_{max}) was reached after only 1 hour (2.1 ± 0.5 μg/ml), while at 10°C and 5°C the C_{max} was reached after 12 hours (5.3 ± 1.7 μg/ml) and 24 hours (3.2 ± 1.8 μg/ml), respectively. Bjorklund and Bylund explained the effect of temperature was due to the fact that fish are poikilothermic, that is their internal temperature varies, often matching the ambient temperature of the immediate environment. Further, an increased ambient and internal temperature should correlate with increased gastric emptying and metabolism in poikilothermic fish species. Therefore, for many farmed fish species in the European Union, the withdrawal times are based on temperature-dependent residue levels and are determined in degree days (Alderman and Smith, 2000). For instance, 150° days for oxytetracycline would represent a withdrawal time of 15 days at 10°C or of 10 days at 15°C.

Distribution

Few studies have investigated the distribution phase of oxytetracycline, with regard to the diffusion of oxytetracycline from the systemic circulation into fish tissues and body spaces (peripheral compartments). Researchers have primarily used the intravenous route of administration in such investigations. Black et al. (1991) calculated a rapid distribution half-life of only 0.9 hours after a single intravenous bolus dose in rainbow trout. Similarly, Rigos et al. (2003) calculated a distribution half-life of only 2 hours in gilthead sea bream. Interestingly, there appears to be little difference in oxytetracycline distribution half-lives in fish and those reported in humans (Gerding et al., 1996). The ability of oxytetracycline to rapidly distribute into the tissues and body spaces where a given pathogen may be targeted, along with its affordability, is part of the justification for its historically widespread use in aquaculture.

Metabolism

Tetracyclines are not metabolized in vivo, but rather are excreted predominantly unchanged in the urine (50–80% of the given dose) (Gerding et al., 1996). Oka et al. (1989) showed that tetracyclines photodecomposed easily in an aqueous solution comparable to a fish pond. Interestingly, Halling-Sorensen et al. (2002) reported other known oxytetracycline degradative products to have antimicrobial activity at levels close to that of their parent compounds. These compounds have largely been ignored in quantitative PK studies due to the minimal metabolism in vivo of tetracyclines, but may need to be considered in future studies.

Elimination

Results of studies on the elimination phase of oxytetracycline from the muscle after oral administration show an obvious decrease in the elimination half-life ($T_{1/2\beta}$) in higher water temperatures. In muscle, $T_{1/2\beta}$ values ranged from 600 hours in brook trout at 7°C (Herman et al., 1969) to 46 hours in perch at 20°C (Wang et al., 2004). Similarly, reported values of $T_{1/2\beta}$ in plasma, serum, and whole blood follow the same temperature-dependent trend as observed in muscle. Haug and Hals (2000) calculated a $T_{1/2\beta}$ of 578 hours in plasma of arctic char held at 6°C in freshwater. Whereas Rigos et al. (2004) calculated a $T_{1/2\beta}$ of 21 hours in plasma of sea bass held at 22°C in saltwater. Poikilothermy is the probable cause of piscine ambient temperature-dependent changes in oxytetracycline elimination. A few inconsistencies in the literature can be found however. Jacobsen (1989) calculated an estimated $T_{1/2\beta}$ of 48 hours from a study conducted in rainbow trout held at 12°C. This value is more than 400 hours less than that observed in another study conducted in rainbow trout held at 11°C (Abedini et al., 1998).

Sulfadimethoxine–ormetoprim

Pharmacokinetic parameters have been determined individually for sulfadimethoxine and ormetoprim in rainbow trout (Droy et al., 1990; Kleinow and Lech, 1992) and channel catfish (Michel et al., 1990; Squibb et al., 1988) using intravenous and oral administration. Pharmacokinetic investigations of the two antimicrobials (Romet®) administered orally together have been conducted in hybrid striped bass (Bakal et al., 2004), Atlantic salmon (Samuelsen et al., 1995), channel catfish (Milner et al., 1994), chinook salmon (Walisser et al., 1990), and rainbow trout (Droy et al., 1989). As with many different pharmacokinetic assessments in fish species, researchers have used several different water temperatures making direct comparisons of data problematic. Temperature-related differences have significant impacts on absorption and excretion kinetics even within the same species (Borgan et al., 1981).

Absorption

Bakal et al. (2004) provided the most complete investigation into the absorption of sulfadimethoxine and ormetoprim when administered in combination. After intraperitoneal (IP) injection in hybrid striped bass at a ratio of 5 : 1, sulfadimethoxine to ormetoprim, the $T_{1/2\alpha}$ was 5.4 hours and 0.7 hours respectively. After oral administration the $T_{1/2\alpha}$ was 3.9 hours and 0.2 hours, respectively. The slower absorption rate after IP administration compared to oral administration may be attributable to the increased surface area enhanced by the peristaltic movements of the GI tract. Also sulfadimethoxine is more soluble in an acidic environment, which could allow for more rapid uptake of the antimicrobial from the stomach. Bakal et al. also calculated a low bioavailability (4.6%) for sulfadimethoxine, indicating poor absorption from the GI tract of hybrid striped bass. Although the 5 : 1 ratio of sulfadimethoxine-ormetoprim is commonly used in feed formulations, Bakal et al. found it does not represent the actual antimicrobial ratio found in the plasma or serum of animals.

Due to the differing absorption rates described here, this antimicrobial combination does not exist in a constant ratio within the animal. To complicate the issue even further, in vitro susceptibility determinations are typically conducted using a ratio of 20 : 1 (Miller et al., 2005), which has been proven to be the optimal ratio of synergism of sulfonamides and their potentiators (Mandell and Sande, 1990).

Bakal et al. (2004) proposed using the total amount of the antimicrobial actually absorbed (area under the concentration–time curve, AUC) in the ratio calculation. This allowed the more close approximation of the average ratio of the antimicrobials in the animal. After oral administration in the hybrid striped bass, Bakal et al. calculated a ratio of 2.14 : 1 (sulfadimethoxine : ormetoprim) based on the AUC for each compound. The relationship of these in vivo-derived ratios to the ratios used in in vitro susceptibility testing is still unclear. How in vivo-derived ratios may be used to help predict therapeutic efficacy for a pathogen with a different MIC ratio also remains to be seen.

Samuelsen et al. (1995) aimed to investigate the bioavailability and pharmacokinetics of sulfadimethoxine and ormetoprim in combination in Atlantic salmon held in 10°C seawater. They showed a considerably higher bioavailability of 39% for sulfadimethoxine and 89% for ormetoprim. Compared to the 4.6% calculated by Bakal et al. (2004) in hybrid striped bass held in 16–17°C freshwater, this exhibits the inconsistency of data present in the literature.

Distribution

Several researchers have investigated the distribution into tissues of sulfadimethoxine and ormetoprim when administered alone in aquatic animal species. Kleinow and Lech (1988) and Squibb et al. (1988) reported comprehensive pharmacokinetics, low protein binding, and wide distribution of radiolabeled sulfadimethoxine following oral and intravenous administration in rainbow trout and channel catfish, respectively. However, only Samuelsen et al. (1995) monitored rainbow trout tissues for the presence of the two compounds after simultaneous administration. They found the sulfadimethoxine and ormetoprim volumes of distribution at steady state to be 0.39 and 2.48 l/kg, respectively. This suggests a much wider distribution of ormetoprim into the tissues outside the plasma than what was seen with sulfadimethoxine. The distribution volume for sulfadimethoxine was similar to that reported previously in experiments involving the single compound: 0.422 l/kg (Kleinow and Lech, 1988), 0.622 l/kg (Squibb et al., 1988), and 0.40 l/kg (Michel et al., 1990). Considerably higher distribution volumes have been reported by researchers for ormetoprim, 4.854 l/kg in rainbow trout (Droy et al., 1990) and 5.503 l/kg in catfish (Plakas et al., 1990). Samuelsen et al. (1995) also noted the kidney had the highest ormetoprim concentration of any organ tested. In salmonids, the kidney contains cells rich in melanin (melanomacrophages). They postulated ormetoprim may bind to the melanin in these cells and could explain the high concentration and prolonged $T_{1/2\beta}$ of ormetoprim in kidney compared to the other organs.

Metabolism

Uno (1993) showed both sulfadimethoxine and sulfamonomethoxine were metabolized by rainbow trout to the N_4-acetylated conjugate, which was the major metabolite, and the N_1-glucuronide conjugate and N_4-acetyl-N_1-glucuronide double conjugate, which were present in lesser amounts. Kleinow et al. (1992) monitored sulfadimethoxine and N_4-acetylated sulfadimethoxine in several tissues and plasma of rainbow trout. They found 20 hours after administration the majority of compound in the plasma was the parent sulfadimethoxine. Conversely, N_4-acetylated sulfadimethoxine predominated in the bile. Slightly higher levels of N_4-acetylated sulfadimethoxine were found in the liver. These findings agree with Squibb et al. (1988) who reported that approximately 90% of the biliary sulfadimethoxine occurred as N_4-acetylated sulfadimethoxine in catfish. These findings also support the view that liver enzymes are instrumental in the extraction of the N_4-acetylated sulfadimethoxine compound from the parent sulfadimethoxine. Plakas et al. (1990) found several unidentified ormetoprim metabolites in the urine of catfish.

Elimination

Samuelsen et al. (1995) showed a fairly rapid elimination for both sulfadimethoxine and ormetoprim, as the elimination half-life, $T_{1/2\beta}$, was 9.9 and 25.6 hours, respectively. In channel catfish $T_{1/2\beta}$ for sulfadimethoxine and ormetoprim was found to be 12.6 and 12.8 hours (Michel et al., 1990; Squibb et al., 1988), whereas Droy et al. (1989, 1990) calculated $T_{1/2\beta}$ for sulfadimethoxine and ormetoprim in rainbow trout to be 16.1 and 17.5 hours, respectively.

Florfenicol

Florfenicol is a relatively new antimicrobial agent to aquaculture, when compared to oxytetracycline and sulfadimethoxine-ormetoprim, which have been used for decades in aquaculture worldwide. Researchers only began studying the PK of florfenicol in fish in the mid-1990s. PK investigations of florfenicol have been conducted in Atlantic salmon (Horsberg et al., 1994, 1996; Martinsen et al., 1993), cod (Samuelsen et al., 2003a), channel catfish (Wrzesinski et al., 2006), rainbow trout (Pinault et al., 1997), and koi and three-spot gourami (Yanong and Curtis, 2005).

Absorption

Horsberg et al. (1996), Martinsen et al. (1993), and Samuelsen et al. (2003a) showed florfenicol absorption after oral administration to be fast and complete with T_{max} and bioavailability values of 6 hours and 99%, 10.3 hours and 96.5%, and 7 hours and 91%, respectively. After oral administration Yanong and Curtis (2005) estimated a $T_{1/2\alpha}$ in koi and three-spot gourami to be 1.4 and 0.6 hours, respectively. After IM administration they calculated a $T_{1/2\alpha}$ of 3.5 and 0.1 hours, respectively.

Distribution

Virtually identical volumes of distribution at steady state were observed by Horsberg et al. (1996) (1.12 l/kg) and Martinsen et al. (1993) (1.32 l/kg) in Atlantic salmon, and Samuelsen et al. (2003a) (1.1 l/kg) in cod. These values indicate florfenicol distributes throughout the body in both species and suggests tissue concentration may be similar to those found in plasma.

Metabolism

Metabolism studies of florfenicol have identified florfenicol amine as the major metabolite in muscle tissue although florfenicol parent is more predominant in skin (FDA, 2005). Florfenicol amine lacks antimicrobial activity, but serves as the marker residue. From 48 hours after administration and throughout a study by Horsberg et al. (1996), florfenicol amine was found in higher concentrations in Atlantic salmon plasma than florfenicol. Samuelsen et al. (2003a) noted a considerable difference in the $T_{1/2\beta}$ of florfenicol after oral administration in cod (39 hours), when compared to that observed by Horsberg et al. in Atlantic salmon (14.7 hours). A negligible temperature difference was cited as a possible explanation; however, no florfenicol amine was detected in either plasma or tissues of cod. The apparent lack of this metabolic pathway in cod may have contributed to the slower elimination of florfenicol and its amine in cod than in Atlantic salmon. However, such a difference in $T_{1/2\beta}$ has not been shown in previous studies with cod and other antimicrobial agents (Samuelsen et al., 2003a; Elema et al., 1994; Rogstad et al., 1993). Metabolism of florfenicol presumably occurs in the liver but this has not been shown experimentally.

Elimination

With the exception of the longer $T_{1/2\beta}$ of cod, florfenicol elimination is quite rapid compared to most other antimicrobials used in aquaculture. Martinsen et al. (1993) and Horsberg et al. (1996) calculated $T_{1/2\beta}$ in Atlantic salmon to be 12.2 and 14.7 hours, respectively. The rapidity of absorption and subsequent elimination in most species has contributed to the attractiveness of florfenicol in aquaculture.

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54

Zoological Pharmacology

Robert P. Hunter

Introduction

Species differences in drug absorption, distribution, metabolism, and excretion (ADME) for numerous pharmaceutical agents have been well documented for domestic species; however, there is limited information concerning the ADME of drugs in nondomestic species. Lack of approved pharmaceutical agents and/or pharmacokinetic data in the literature for exotic, wildlife, and zoo species is a major issue for veterinarians attempting to treat these animals. Under the Animal Medicinal Drug Use Clarification Act (AMDUCA), practitioners take approved agents (veterinary or human) and extrapolate their use to nonapproved species, often with little scientific basis to support this decision. To further complicate treatment, zoo veterinarians often have to formulate the medication(s) into a meal or treat, hoping that the animal will ingest it. Due to lack of patient compliance, the clinician may have to resort to other means of drug administration. Additionally, due to the value of these animals or their status as threatened or endangered species, the traditional method of trial and error for treatment selection is inappropriate. This results in a mentality where no zoo veterinarian wants to be the first to administer an agent/formulation in an untested species.

The range of animals a zoo veterinarian cares for varies from very small invertebrates (honeybees) to megavertebrates such as elephants and whales. There is currently only one United States approval for the class amphibians, four agents approved for use in invertebrates, and eight to ten compounds for mammalian zoo and wildlife species compared to almost 300 for domestic cattle. Various methods have been used to extrapolate/predict safe and effective dosage regimens. No attempt will be made to turn this chapter into a formulary. Excellent formularies already exist to provide for that need (Antinoff et al., 1999; Carpenter, 2012; Hawk et al., 2008; Kreeger and Arnemo, 2007; Nielsen, 1999). This chapter provides information and guidance on comparative physiology, formulation considerations, and dosage scaling across wide and varied species.

Species Groups

Working with exotic animals, even during routine daily care, can be very dangerous. This is magnified when working around sick or injured animals where the patient is subject to increased stress, pain, and deviations from the usual routine — for example, those associated with treatment and drug administration. If given the opportunity, most animals can inflict injury to humans using almost any body part. They can crush with the body or head; kick with exceptional force; or grasp with their hands, body, tail, or trunk. During medication attempts, the individuals administering the treatment are at great risk because they are close to the animal and often working near the head, teeth, fangs, or hands. Safety precautions must be considered prior to treating any animal.

Invertebrates

The numbers of invertebrate zoos and species within those facilities are increasing rapidly. The main concern when treating these species is that they typically lack the membrane barriers that are foundations for therapeutic choices in domestic mammalian species. This likely makes them more susceptible to serious adverse events from agents that are considered safe in mammals, especially anthelmentics or neuroacting agents.

There are only four agents approved for use in invertebrates (shellfish, shrimp, lobsters, or honeybees): bicyclohexylammonium, formalin, oxytetracycline, and tylosin. All of these species are treated as food animals and residues are of concern. These agents have specific approvals in one or more of these species.

Another consideration is the use of invertebrates as drug delivery devices. This is typically done for insectivores or marine mammals (squid to sperm whales). The book *Eat this Bug*, by Davis (1996) may be useful for assisting veterinarians in selecting appropriate “treat” species to use as drug vehicles. This concept is similar to the use of rodents as dosage forms (“fuzzy” dosage forms) for raptors or reptiles. This concept of using insects as

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dosage forms is commonly used clinically to increase calcium in the diet of insect-eating reptiles (Donoghue and Langenberg, 1996).

Vertebrates

Reptile and Amphibian

With over 4,000 amphibian species, 3,750 species of lizards, 2,500 species of snakes, 270 species of turtles, and 22 species of crocodilians there is currently only one approved product in the USA for this group. Tricaine methanesulfonate (MS-222) is a Na^+-channel–blocking local anesthetic. It is used for temporary immobilization to aid in handling during weighing, measuring, marking, surgical operations, transport, photography, and research.

Route of administration is especially challenging in these species. For amphibians, oral, topical, and injectable routes are common. Topical baths can be very beneficial since the skin of amphibians has similar properties to mammalian mucosal surfaces. The pH and temperature of the bath must be appropriate for the treated species (Mader et al., 1985; Walker and Whitaker, 2000). This can also allow for group treatment of animals that are on display or for treatment off-exhibit in special treatment tanks. As with all treatments, the animals must be monitored so that they are not harmed or injured in the bath (Walker and Whitaker, 2000).

Routes of drug delivery are similar between reptiles and amphibians. Several studies have been done investigating the pharmacokinetics of various agents in all four groups of reptiles: snakes, turtles, lizards, and crocodilians.

Drug metabolism may be affected by prandial state in the snake and other sporadic feeding reptiles. This mechanism allows for minor metabolic investment of digestion to occur after a period of fasting and energy reserve depletion; thus nutrient metabolism peaks about 1 week after feeding. With variable physiological states, drug metabolism and elimination could be affected by feeding intervals. Thus, the effect of feeding could greatly affect the way orally administered pharmacological agents are absorbed by snakes. When a snake consumes a meal, the small intestinal mucosa will increase in thickness at least three times while total length of the small intestine does not change. Correspondingly, villi length is two times prefeeding length, resulting in an increase in small intestine surface area as a result of feeding. Azithromycin absorption in ball pythons was prolonged, compared to all mammalian species reported, based on relatively large mean absorption time (MAT) and T_{max} values.

Four of the 15 circulating azithromycin metabolites: 3′-desamine-3-ene-azithromycin, descladinose dehydroxy-2-ene-azithromycin, 3′-desamine-3-ene descladinose-azithromycin, and 3′-*N*-nitroso,9a-*N*-desmethylazithromycin are unique to the ball python. As azithromycin metabolism in mammals is mediated by several cytochrome P450 isoforms, it is possible that these systemic metabolites are a result of novel cytochrome P450 isoforms, the unique gastrointestinal physiology of the ball python, or some combination of both. The unique gastrointestinal physiology of the snake does appear to lend itself to increased metabolism of xenobiotics. Due to the long GI transit times of boid snakes of >1 week, this presents the opportunity for a xenobiotic, such as azithromycin, to undergo repeated enterohepatic recirculation during the course of a single drug administration. This provides an effect similar to that produced by sustained-release oral formulations in mammals. The percentage of each plasma metabolite differed for only a few metabolites when comparing intravenous with oral administration results from this study (Hunter et al., 2003b). This phenomenon would be expected to correspondingly prolong tissue concentrations of the parent compound and its metabolites. These factors are also likely to have an impact on bioavailability relative to time of feeding in snakes. This would likely make prediction of oral drug absorption dependent, to some extent, on time after feeding.

Reptiles have a renal portal system. This means that a first-pass effect of renally cleared drugs can take place if the animal is injected in the posterior half of the body. This system is not fully understood, and it may not be as restrictive as once believed. However, until definitive data are available, reptiles should be injected in the anterior portion of the body when agents dependent upon renal clearance are administered.

The rehabilitation of wild reptiles, such as sea turtles, requires the use of a variety of different medications; most have never undergone any pharmacokinetic studies in the target species to establish a basis for a safe and efficacious dose. Many of the commonly used treatments have had few studies (pharmacokinetic or efficacy) in any reptile species. This makes use of these drugs guesswork at best and dangerous at worst. For instance, when antimicrobials are underdosed this may not only lead to ineffective treatment of bacterial infections but may also lead to the development of resistant strains of bacteria. Likewise, overdosing may lead to adverse effects. The low basal metabolic rate of reptiles, in general, may correlate to the overall metabolism and distribution of drugs; this, along with the variation in metabolic rates among reptile species, makes dosage determination from dramatically different reptile species far less than desirable — let alone when extrapolation from mammalians species is done. The extent of plasma protein binding varies between species and is temperature dependent. This is a key point to remember when treating poikilothermic species.

Avian

Avian medicine is a field of rapid growth in veterinary medicine. Greater than 95% of all pharmaceuticals used in clinical practice to treat various avian species are based upon empirical doses and the personal experiences of the attending clinician. This raises issues concerning the health of the animals being treated. Therefore, the pharmacokinetics of pharmaceutical agents used to treat avian species is an area of research that is needed to ensure the safe and proper dosing of these animals. However, there is limited information on the pharmacokinetics of agents used to treat psittacines, anseriforms, galiforms, passerines, softbills, and raptors. As a result of the lack of pharmacokinetic data, dosage regimens are designed using linear extrapolation from doses approved in domestic species, such as poultry (Baert and De Backer, 2003). It is possible that use of linear extrapolation could lead to the development of toxicity or lack of efficacy in the treated animal(s) as a result of inaccurate dosing.

Extrapolation of doses is difficult between mammalian and avian species, both qualitatively and quantitatively. The avian renal cortex is more similar to the reptile cortex than the mammalian cortex. Glomerular filtration is not constant in birds, as it is in mammals, and likely has an impact on the pharmacokinetics of pharmaceuticals in avian species (Frazier et al., 1995). Avian species appear to be more susceptible to the renal ischemia and tissue damage of nonsteroidal antiinflammatory drugs (NSAIDs) than the gastrointestinal side effects (Paul-Murphy and Ludders, 2001). While avian cytochrome P450 activities have been reported, their expression and role in avian drug metabolism is not well documented (Walker, 1998).

All of the approvals in this group are for species used as food animals (poultry and galliforms). While it would seem logical to extrapolate a dose from a chicken to an ostrich, this could be equated to extrapolating a dose in horses to elephants.

The concept of "fuzzy" (use of rodents) dosage forms for raptors has been mentioned previously. While rodents can be viewed in many ways (pets, pests, or research test subjects), to the avian (and reptile) veterinarian they are a drug delivery device. In red-tailed hawks, the extent of oral bioavailability of enrofloxacin between intramuscular and "fuzzy" oral dosing was not different, even though the oral T_{max} was five times the intramuscular route (Harrenstien et al., 2000). The difference in T_{max} appears logical when considering dissolution and gastrointestinal transit in these animals.

Mammalian

Oral absorption has been shown to vary within a species. Within domestic dog breeds (Beagle and mongrel), different gastrointestinal transit times and mouth-to-small-intestine transit times have been reported (Sagara et al., 1995). When one considers the anatomical differences between true monogastrics (canine or feline species), hindgut fermentors (rodents, rabbits, horses, or elephants), foregut fermentors (Colobus monkeys, camelids, and kangaroos), and ruminants (cattle, goats, sheep, or antelope), the potential differences are staggering and support the concept that bioavailability is not scalable across mammalian species (Mahmood, 2000, 2002). The large body sizes of some mammalian species produces problems for treatment and places significant limitations on drug delivery. Aside from the weight of the animal, the size, thickness, and density of various anatomical structures can physically hinder drug administration. These concepts are discussed in Chapter 2 of this text.

Small/Pocket Pets/Rodents

There is a wealth of data in the literature concerning the pharmacokinetics and safety of almost every human drug in rodents, such as mice and rats and often for rabbits as well. While ferrets are a laboratory species, research is narrower in scope, but could still be useful in treating these small mammal species. While the doses may be above that needed for efficacy, safety is well understood. The issue is typically formulating the agent into a dosage form that can be used clinically. With the Internet and various medical literature search tools, such as Medline, PubMed, and Highwire, the busy clinician can find information for treating just about any disease in small mammals.

However, there is no published, peer-reviewed information concerning the pharmacokinetics of drugs used in more exotic small mammals, such as hedgehogs, sugar gliders, and honey bears. Historically, mite infestations (*Chorioptic* sp.) in hedgehogs have been treated with either ivermectin or amitraz (Lightfoot, 2000). This treatment has been shown to be effective, but requires weeks or months of therapy. Fleas and lungworms also infest these animals, and with a broad-spectrum, topical endectocide, such as selamectin, treatment could be carried out easily and rapidly, allowing for treatment of both the mite and flea infestations with a single compound (Figure 54.1).

Farmed/Domestic Species

There are ≤50 compounds approved in the United States for sheep, ≤12 for goats, and none for New World camelids. As producers and veterinarians have taken approved agents for cattle, sheep, or goats and extrapolated their use to exotic domestic species, such as New World camelids, safety and efficacy have been the main issues. There are many different species-related factors, including absorption, elimination, and metabolism, that

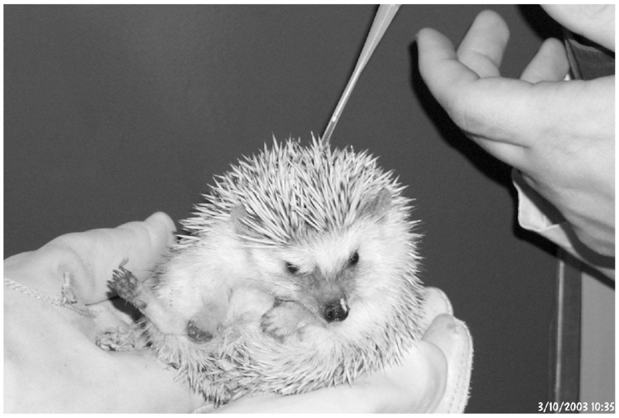

Figure 54.1 Topical dose administration to an African hedgehog.

could lead to variation in the pharmacokinetic parameters between species and possibly decrease the efficacy or increase the toxicity of a compound (Hunter and Isaza, 2002; Short, 1994; Xia et al., 2006). These differences make extrapolation very difficult from one species to another.

An example of species differences in pharmacokinetics is the data for topical doramectin. The mean C_{max} and AUC values for llamas and alpaca was approximately 33% less than that reported for cattle using the same dose and formulation. The mean T_{max} values for both species were 1 day longer than reported in cattle. It appears that the extent of absorption, as evaluated by C_{max} and AUC, is much less than that reported for cattle (Hunter et al., 2004a). This was also the case for moxidectin in these two species (Hunter et al., 2004b).

Zoo/Marine Mammals

All of the routes and issues discussed previously apply to this group of patients. If a well-trained animal can be difficult to treat (which they are), an uncooperative animal can easily prevent almost any unwanted medication from being administered. This is especially true of large carnivores, primates, and megavertabrates (elephants). The animal's combined size, strength, and/or intelligence effectively precludes forcing it to ingest medication or allowing someone to get close enough to manually administer medication.

The capture and chemical immobilization of a variety of zoo and wildlife species has been enhanced with the use of potent synthetic opiates, such as carfentanil (Haigh, 1990). These opiates generally provide rapid induction and relatively long-lasting immobilization. They are usually administered parenterally, the small volumes required making them ideally suited for use in remote delivery systems such as darts (Fowler, 2008). Furthermore, immobilization by these drugs can be completely reversed by the administration of specific opioid antagonists. This is particularly important for immobilization of free-living wildlife, which cannot be closely monitored after the immobilization period. These opioid anesthetic agents have provided zoo veterinarians and wildlife researchers not only a method to restrain animals for a variety of medical and research procedures, but have become an indispensable tool to the practice of zoological veterinary medicine.

Carfentanil has become the drug of choice for a wide variety of nondomestic animal immobilizations, particularly for hoofstock, and has been used either alone or in combination with sedatives for rapid immobilization of captive and free-ranging wildlife. The potency and formulation of this drug allow for a small volume to be administered, which is ideal for remote delivery systems (darts). Naltrexone is the antagonist used routinely in carfentanil immobilization procedures, because it has a longer half-life than other antagonists such as naloxone or nalorphine in domestic species. One adverse effect with the use of carfentanil is that of renarcotization. This is the reoccurrence of the effects of the opioid agonists up to 72 hours after apparent full recovery from the drug. The effect varies in severity from mild sedation to full immobilization and has also been associated with a high incidence of mortality. The choice of antagonist and dose used are important factors in the incidence of renarcotization. Also, certain species appear to have a higher tendency to develop renarcotization than others. Possible mechanisms for renarcotization are listed by Haigh (1990): enterohepatic recycling, metabolism of antagonist to form a metabolite with agonist properties, release of the agonist from body depots after the antagonist has been eliminated, antagonist underdosing, and metabolism of a short-acting antagonist and reestablishment of a longer-acting agonist. Studies are needed to assist with determining the actual cause(s) but there are currently no published PK/PD studies of any opioid agonist or antagonists in exotic ungulates.

Anesthesia of nonhuman primates is risky and stressful. Squeeze cages and darts have been used for administration of parenteral anesthetic agents, and various anesthetics have been administered orally using treat or food items. Although these formulations work well for these primate species, care should be taken to avoid conditioning the animal to associate the treat or personnel with the procedure (Hunter and Isaza, 2002). Transmucosal fentanyl is approved for use in the United States as a preanesthetic agent in humans in a lollipop formulation. When this preparation of fentanyl is chewed and swallowed, it still results in a sedative effect with a corresponding longer T_{max} when compared to true transmucosal absorption. When this formulation was used in a study on great apes (Figures 54.2, 54.3), nine chimpanzees were evaluated with only one being successfully offered a dose

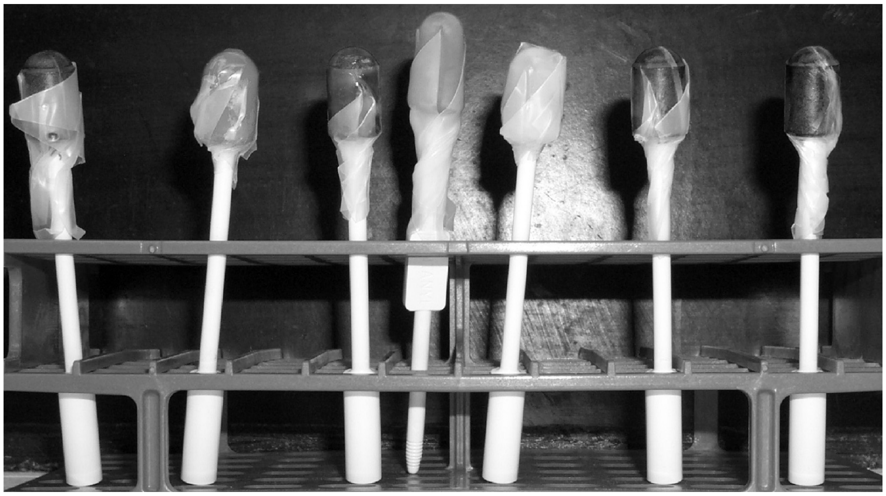

Figure 54.2 The white fentanyl (Actiq®) lollipops were painted with candy coloring to match the colors of the placebo lollipops.

after having refused the lollipop on an earlier attempt; two other animals also refused to ingest their lollipops. All orangutans accepted at least one dose with several accepting two or more. The gorillas accepted multiple lollipops. The orangutans and gorillas demonstrated less stress when darted and showed signs of sedation after fentanyl administration. The main metabolite in humans, norfentanyl, was identified in only one of the test subjects, an orangutan. An effective dosage of fentanyl for orangutans and gorillas was dependent on an effective training program; cooperation of the keeper staff and zoo administration; and facilities that allow for individual, overnight housing of animals. In contrast, chimpanzees were suboptimally sedated with transmucosal fentanyl (Hunter et al., 2004c).

Foot problems affect over 50% of the captive elephant population. These problems may progress to arthritis, a debilitating condition often resulting in euthanasia of the animal. Arthritic elephants are typically treated with a wide variety of antiinflammatory and analgesic drugs, which offer only palliative treatment. Due to the side effects of these agents in domestic species, accurate therapeutic regimens are critical to properly treating diseased elephants. Simply guessing at a dosage can have disastrous consequences, as was illustrated in a case report where lysergic acid diethylamide (LSD) was tested in an

Figure 54.3 Use of fentanyl lollipops to sedate an orangutan.

elephant and the animal died violently within minutes (West et al., 1962).

The lack of pharmacokinetic data is reflected in the sparse number of published studies in elephants. Almost all of these studies have focused on antimicrobials, with little data being reported for analgesic and/or antiinflammatory agents. As a result of the lack of pharmacokinetic data, dosage regimens are designed using the assumption that pharmaceuticals administered to the elephant are absorbed, distributed, metabolized, and excreted in approximately the same manner as horses (Page et al., 1991). However, at least one example exists in the published literature to contradict this statement. For the chiral NSAID ketoprofen, the *S*-enantiomer is cleared from elephant plasma much more rapidly than the *R*-form. This is different than the domestic horse where the *R*-form is cleared more rapidly. This was explained as either selective clearance from plasma in the Asian elephants or as chiral inversion. Also in this study a possible novel metabolite, a Phase II glycine conjugate, was reported (Hunter et al., 2003a).

Allometry in Zoo Medicine

Allometric scaling of pharmaceuticals to predict pharmacokinetics in zoo/exotic animals has considerable benefit for zoological veterinarians. This tool, when used appropriately, can provide an *estimate* for designing dosage regimens. This topic was discussed for minor species in Chapter 51 of this text. The example of differences in ketoprofen inversion across species emphasizes the need to understand and be aware of the assumptions when designing treatment regimens based on allometric scaling data.

Just as mammals can range from a few grams to thousands of kilograms, reptiles and birds can also vary in body weight across a wide range. It has been suggested that it is impossible to derive a single equation correlating body mass to metabolic rate for all 6,000 species of reptiles (Funk, 2000). Without knowledge as to the extent and route of elimination of an administered pharmaceutical agent, extrapolation of dosage regimens from one class to another is difficult, if not impossible, with any certainty. It is apparent that more research is needed on the drug metabolism and excretion capabilities in snakes, let alone reptiles in general. This type of research would increase the ability of clinicians to determine more effective and safe dosage regimens for their patients. Another consideration when using allometry is the nonlinearity of pharmacokinetics in some species (Manire et al., 2003; Rush et al., 2005).

There are two major components of hepatic clearance of xenobiotics: hepatic blood flow and enzymatic metabolism. Renal clearance is the other major route by which drugs are excreted. Renal clearance is composed of three parts: glomerular filtration, active tubular secretion, and tubular reabsorption. Glomerular filtration rate is allometrically scalable in mammalian species, as is *p*-aminohippuric acid clearance (Edwards, 1975; Holt and Rhode, 1976). However, allometric scaling of active tubular secretion and tubular reabsorption have not been reported in the published literature.

The concept of metabolic or allometric scaling has been used as the basis for comparison across species since the 1930s. Benedict's *Vital Energetics* appears to be the first comprehensive discussion on the topic of metabolic scaling. Benedict's work laid the foundation for scaling of metabolic rates across a wide range of species. Metabolic rate for mammals was described by Kleiber (1932, 1961). Various physiological processes such as heart rate, blood volume, and glomerular filtration rates can be predicted reasonably accurately for a wide variety of species based solely on the weight of the animal.

Linear extrapolation is the use of a single mg/kg dose across all species, so that the total amount of drug increases in a linear fashion as body weight increases. When dosages are extrapolated to other species of different weights, this method assumes that dosage versus weight is directly (linearly) proportional. This method assumes that differences in weight and in species pharmacokinetics are not clinically relevant. Although this is the simplest and most common method of extrapolation, it may overdose large animals and underdose small animals.

Metabolic scaling uses the ratio of a known physiological process (i.e., metabolic rate) of two species to estimate a dosage in a species where there are no measured pharmacokinetic data. This method uses the dosage in a specific species and links the dosage to a physiological function instead of the animal's body weight (Dorrestein, 2000; Mortenson, 2001; Sedgwick, 1993; Sedgwick and Borkowski, 1996). In this method the chosen function forces an allometric (log–log) relationship, usually with an exponent of 0.6–0.8. The key assumption is that since most physiological functions follow allometric equations relative to body weight, pharmacological parameters must also follow similar allometric relationships, and, to this end, basal metabolic rate is used as the universal scaling factor. This method of dose extrapolation provides a relatively smaller dose for large animals and a higher dose for small animals. A specific method for interspecies extrapolation of drug dosages used in the zoological medicine field is metabolic scaling as described by Sedgwick and others (Dorrestein, 2000; Mortenson, 2001; Sedgwick, 1993; Sedgwick and Borkowski, 1996). All species are placed in one of five groups, termed *Hainsworth's energy groups:*

passerine birds, nonpasserine birds, placental mammals, marsupial mammals, or reptiles. Using a predetermined K value, a specific minimum energy cost (SMEC) value is calculated and the ratio of the target species SMEC to the SMEC of a safe effective dose in a known species is calculated to achieve that appropriate dosage regimen (Dorrestein, 2000; Mortenson, 2001; Sedgwick, 1993; Sedgwick and Borkowski, 1996). This method is simple in that the metabolic rate can be estimated for most species and applied to any pharmaceutical agent. A known drug dosage is multiplied by the ratio of metabolic rate of a known, similar species and the new species. Unfortunately, this method has not been well validated and several pharmacological papers have illustrated specific failures in this method of extrapolation.

The allometric approach is to measure a pharmacokinetic parameter in multiple species and to plot the data against weight to derive a new allometric equation that can be used to estimate the pharmacokinetic parameter in an unknown species. Similar to its use for selecting the first time dose in humans (Boxenbaum and DiLea, 1995), interspecies extrapolation serves an important function within the framework of veterinary medicine. In this regard, among its uses, it is especially important for estimating an appropriate dose in large animal species for which there does not exist pharmacokinetic or clinical information to support dose estimation. The caveat is that where first dose in humans is a prediction of a safe dose, in zoological medicine, the extrapolated dose in nonapproved species must be *both* safe and efficacious. Despite the potential for extrapolation error, the reality is that allometric scaling is needed across many veterinary practice situations and therefore will be used. For this reason, it is important to consider mechanisms for reducing the risk of extrapolation errors that can seriously affect target animal safety, therapeutic response, or the accuracy of withdrawal time predictions. Based upon this analysis, it appears that inclusion of at least one large animal in the scaling (with or without human data) can improve predictions (Mahmood et al. personal communication). In large animals, correction factors could not be applied because there was no trend seen between the exponents of the simple allometry and the appropriate correction factor for improving our predictions (Mahmood et al. personal communication). In addition, the vast majority of large animals for which pharmacokinetic data are available are herbivores. Conversely, the smaller animal species are either omnivores (mouse, rat, monkey, and human) or carnivores (dogs and cats). This difference in feeding behavior itself can influence drug metabolism and renal elimination. While large carnivore data (lion, tiger, polar bear, etc.) would be helpful, the obvious dangers hinder this area of research. Some marine mammal species may be worthy of consideration, but due to body fat composition of species, such as killer whales (*Orcinus orca*), the results would be of limited use. There is little way to predict, a priori, which animal species will be best suited for inclusion in the interspecies predictions. Another point of consideration is the interspecies differences in the contribution of various organ systems and differences in body composition as it pertains to the body weight estimates.

All extrapolation methods assume that the route of elimination is similar across all species used in the extrapolation and this is simply not true. The reality is that most drugs, 75% in Riviere's published data (Riviere et al., 1997), are not scalable across multiple species and that allometric scaling of pharmacokinetic parameters, while useful, has limitations. It is necessary, for the best therapeutic outcome, to have pharmacokinetic and efficacy data in each species that is treated in zoological medicine. The use of the available literature to understand the route of elimination and the extent of metabolism of therapeutic agents will greatly assist in determining allometric relationships of pharmacokinetic parameters. This should lead to more realistic and rational design of dosage regimens in zoological medicine.

"No presently available chemical restraint agent is equally effective and safe for use with all 45,000+ vertebrate species" (Fowler, 2008). This statement relates not just to chemical restraint, but to therapeutic use in general within zoological medicine. Changes in minor species drug approval could make it easier for the needed information to get into the hands of veterinarians. The Minor Use and Minor Species Animal Health Act of 2004 (MUMS) has been enacted to address the lack of drug availability for minor species. This includes food animals, companion pets, wildlife, and zoo species. An increase in basic pharmacokinetic parameters in zoological species will increase the therapeutic options for veterinarians. Any pharmacokinetic or pharmacodynamic studies in these species would be welcomed.

Some of the specific needs for zoological medicine are formulations that would allow for administration of therapeutic agents via the drinking water or milled into the feed. Other routes of administration that have not been used to their full extent in zoological medicine are rectal, sustained or controlled release, and topical. Increased information and formulations that would allow for administration of appropriate therapeutic agents would be of great benefit. This chapter brings to light some of the issues that face veterinarians on a daily basis when they deal with nontraditional species. Clearly it is not economically feasible to develop every drug for every species. However, targeted approvals or research could greatly increase the efficacy and safety of current and future therapeutics. Also, this type of approach would increase the quality of care provided to zoological species.

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MUMsR$_x$: http://www.nrsp-7.org/mumsrx/
Elephant Care International: http://www.elephantcare.org/
Association of Reptilian and Amphibian Veterinarians: http://www.arav.org/Default.htm
American Association of Zoo Veterinarians: http://www.aazv.org/
Association of Avian Veterinarians: http://www.aav.org/
International Association for Aquatic Animal Medicine: http://www.iaaam.org/
MUMS act: http://www.fda.gov/AnimalVeterinary/DevelopmentApprovalProcess/MinorUseMinorSpecies/default.htm

Section 12

Regulatory and Other Considerations

55

The Regulation of Animal Drugs

Steven D. Vaughn and Marilyn N. Martinez

Introduction

This chapter describes the regulatory framework that guides animal drug approval within the US. The regulation of animal drugs is administered by the US Food and Drug Administration (FDA). The FDA is given its authority from the US Congress through the Federal Food Drug and Cosmetic Act (FFDCA). The FFDCA has been amended many times since its original passage in 1938 to provide new and refined provisions, some of which will be discussed in this chapter.

The FDA/Center for Veterinary Medicine (CVM) website is a good reference source to bookmark for keeping abreast of regulatory policies and issues pertaining to the regulation of animal drugs and feeds. The CVM home page can be accessed at: www.fda.gov/AnimalVeterinary. The CVM Homepage also provides links to the Freedom of Information (FOI) summaries for approved New Animal Drug Applications (NADAs), Supplemental NADAs, and Abbreviated New Animal Drug Applications (ANADAs). These summaries provide valuable information on the data submitted to support the approval of the application. The website also hosts a searchable database: Animal Drugs @ FDA.

The availability of approved new animal drugs for use in livestock, poultry, pets, and other animals is vital to protecting the health of animals, increasing the efficiency of food production, and to protecting the health of humans who consume the products of food-producing animals. Appropriate use of animal drugs also enhances human health through prevention of zoonotic disease transmission and protection of the food supply. With regard to companion animals, healthy pets enhance the quality of human life through the human–animal bond. Practical application of the principles of pharmacology is important for development of safe and effective animal drugs and for making the best use of these products during clinical practice. An understanding of considerations and regulatory constraints that guide the evaluation of veterinary pharmaceutical products by the FDA is vital for optimizing the animal drug development process and for using label information to customize treatments to match patient needs.

Drugs versus Biologics versus Pesticides

Animal products are regulated by several agencies. These agencies work together to determine which regulatory authority is the best fit for a particular animal product. The intent of this cooperation is to ensure consistency and equity in the regulation of animal products and to avoid animal products being regulated by multiple authorities and under different laws. FDA/CVM regulates the manufacture and distribution of drugs, food additives, and medical devices used in veterinary species. This includes both product approval and postapproval monitoring to ensure continued product safety and effectiveness.

Animal Drugs

Section 512 of the Federal Food Drug and Cosmetic Act (FFDCA) provides the statutory provisions governing the regulation of animal drugs. Through this act, a new animal drug is considered to be unsafe unless there is in effect an approval of a New Animal Drug Application (NADA) and unless the use of a drug and its labeling conform to the approved application. Any drug that is deemed unsafe is considered to be adulterated within the meaning of the FFDCA.

Device

A device is an instrument, apparatus, implement, machine, contrivance, implant, in vitro reagent, or other similar or related article, including any component, part, or accessory, which is intended for use in the diagnosis

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of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, in man or other animals, or intended to affect the structure or any function of the body of man or other animals, and which does not achieve any of its principal intended purposes through chemical action within or on the body of man or other animals and which is not dependent upon being metabolized for the achievement of any of its principal intended purposes. Currently, there are no FDA premarketing approval requirements for medical devices intended for animal use. However, veterinary medical devices are subject to the general provisions of the Act that relate to misbranding and adulteration. (Sections 501 and 502 of the FFDCA).

Veterinary Biologics

The US Department of Agriculture, Animal and Plant Health Inspection Service, Veterinary Services, Veterinary Biologics regulates veterinary biologics (such as vaccines, antitoxins, and diagnostics that are used to prevent, treat, or diagnose animal diseases) under authority of the Virus Serum Toxin Act of March 4, 1913. The FDA and USDA work together through a Memorandum of Understanding to determine whether a product will be regulated as an animal drug or as a veterinary biologic. The regulations covering veterinary biologics may be found in Title 9 of the Code of Federal Regulations.

Pesticides

The Environmental Protection Agency regulates pesticides (including preparations for use on inanimate objects, rodenticides, and most insecticides) are regulated by the EPA under the authority of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA). The FDA and EPA work together through an informal agreement to determine whether a product will be regulated as an animal drug or a pesticide. Title 40 of the Code of Federal Regulations contains the regulations under which pesticide programs operate.

What is a New Animal Drug?

A product is determined to be a new animal drug by its intended use. An animal drug is a product intended for use in animals for the diagnosis, cure, mitigation, treatment or prevention of disease, or a product other than food, intended to affect the structure or any function of the body of animals. This includes any drug intended for use in animal feed but not including the animal feed. If the animal drug's composition is such that the drug is not generally recognized as safe and effective for the use under the conditions prescribed, recommended, or suggest in the labeling of the drug (21 U.S.C. § 321(v)) and some other criteria, it is deemed to be a new animal drug. Virtually all animal drugs are "new animal drugs" within the meaning of the FFDCA, and are therefore subject to Section 512 of the FFDCA.

As mandated by the FFDCA (the Act), a new animal drug may not be introduced into interstate commerce unless it is:

- the subject of an approved new animal drug application (NADA), or
- the subject of an abbreviated NADA (ANADA), or
- the subject of a conditional approval (CNADA) pursuant to 21U.S.C. §360ccc or
- there is an index listing in effect pursuant to 21 USC § 360ccc-1 (21 U.S.C. §§ 331(a) and 360b(a)).

Under section 512(j) of the act, new animal drugs may be exempt from the approval requirements of the act if they are intended solely for investigational use to evaluate the safety and effectiveness of the drug.

Laws, Regulations, and Guidance

The FDA is authorized by the US Congress to implement certain laws. For example, the predominant statutory authority for FDA to regulate animal drugs is the Federal Food, Drug, and Cosmetic Act. Other laws, such as the Environmental Policy Act, are also used by the FDA. The FDA further interprets these laws by promulgating federal regulations. These regulations have the force of law and are used to further ensure that the intent of Congress is followed. These regulations are published in the Code of Federal Regulations (CFR). The section of the CFR addressing the regulation of new animal drugs is Chapter 21. The FDA will often issue other documents, known as guidance, that provide the Agency's current thinking on a particular subject. These guidance documents are not legally binding on the Agency or a drug sponsor but are helpful in describing to a sponsor how to approach addressing a statutory requirement. Guidance documents are available on the FDA website.

History of the FDA and its Relationship to Veterinary Medicine

Although veterinarians have been a part of the FDA since it was formed in 1927, regulation of veterinary pharmaceuticals was not initiated until the 1950s. When the FDA was divided into five Bureaus in 1954, a branch dedicated to the evaluation of veterinary medicine was created within the Bureau of Medicine. This branch became

Table 55.1 Historical overview of the US FDA. Source: US Food and Drug Administration.

1848: Import Drugs Act (first Federal statute for guaranteeing quality of medicines).
1862: President Lincoln created the Department of Agriculture (USDA).
1880: After a year's investigation into food and drug adulteration, USDA began advocating enactment of a national food and drug law.
1906: First Federal Food and Drug Act was signed into law and was administered by USDA's Bureau of Chemistry after studying food and drug adulteration.
1927: The Food, Drug, and Insecticide Administration was formed.
1938: Due to deaths from a sulfanilamide elixir containing an antifreeze analogue, the Federal Food, Drug, and Cosmetic Act (FFDCA) was enacted, requiring manufacturers to provide evidence of product safety.
1951: Durham-Humphrey Amendment initiated prescription requirements for certain human drugs. The veterinary Rx legend was effected through rulemaking.
1953: The FDA was transferred to the Department of Health, Education and Welfare.
1954: The FDA was organized into five Bureaus, including a Bureau of Medicine, within which was a Veterinary Medical Branch.
1959: The Veterinary Medical Branch became a Division due to extensive use of animal drugs and medicated feeds.
1962: Kefauver-Harris Drug Amendments resulted from the thalidomide disaster, requiring that drugs show both safety and effectiveness, and establishing GMPs.
1965: The Bureau of Veterinary Medicine (BVM) was established.
1984: The BVM became the Center for Veterinary Medicine (CVM).

a bureau of its own in 1965, and, in 1984, the Bureau of Veterinary Medicine became the current Center for Veterinary Medicine (CVM). A brief chronology of FDA history is provided in Table 55.1. Additional information on the history and current responsibilities of the FDA and CVM is available on the website (www.fda.gov).

Other Important Amendments to the FFDCA

Generic Animal Drug and Patent Term Restoration Act

On November 16, 1988, the President signed into law the Generic Animal Drug and Patent Term Restoration Act. The new law, known as GADPTRA, amends the Federal Food, Drug, and Cosmetic Act to provide for the approval of generic copies of animal drug products that have been previously approved and shown to be safe and effective when used in accordance with their labeling. Under GADPTRA, a generic animal drug product may be approved by providing evidence that it has the same active ingredients, in the same concentration, as the approved animal drug product, and that it is bioequivalent to the approved animal drug product.

The GADPTRA provides for a period of 3 years of marketing exclusivity for a new use of an animal drug (a use that required reports of new clinical or field investigations for its approval), during which time no abbreviated application for a generic copy may be approved for the new use. The law also provides for a period of 5 years of marketing exclusivity for an animal drug that has not been previously approved in any new animal drug application. During this period, no abbreviated application may be submitted. (Exception: An abbreviated application may be submitted after 4 years if the generic applicant claims noninfringement of a listed patent that is claimed for the approved product or its use.) The law also provides for another form of marketing exclusivity, known as patent term restoration. This type of exclusivity extends the period of protection by US patent for an animal drug, or its method of use, that was approved after November 16, 1988, to compensate for the time that was required for investigation and regulatory review of the animal drug prior to its approval. Patent term restoration is not related to the exclusivity periods described above and may overlap those exclusivity periods.

The information necessary to obtain approval to market a generic animal drug is submitted to FDA in the form of an Abbreviated New Animal Drug Application (ANADA). The application must provide certification by the applicant that a patent does not exist, that a patent has expired or will soon expire, or that a patent claimed for the approved product is invalid or will not be infringed upon by approval of the abbreviated application. In the latter case, the generic applicant or sponsor must then notify the sponsor of the approved product application and the owner of the patent that he or she has filed an abbreviated application claiming invalidity or noninfringement of the patent.

All animal drugs that were approved for safety and effectiveness on November 16, 1988, or have been approved since that date, and are not protected by patent or exclusivity are eligible for copying under the provisions of the GADPTRA, unless the animal drug has been subsequently withdrawn from the market for safety or effectiveness reasons, or unless it is the subject of a Notice of Hearing that has been published in the Federal Register. To meet requirements of the GADPTRA, the Center for Veterinary Medicine has arranged for the publication of a list of animal drug products that are eligible to be copied as generics (Green Book).

The Animal Medicinal Drug Use Clarification Act of 1994

The Animal Medicinal Drug Use Clarification Act (AMDUCA) enabled veterinarians for the first time to use a human or animal drug in an extralabel manner under certain conditions. Prior to the enactment of the AMDUCA of 1994, the FFDCA prohibited veterinarians from prescribing new animal drugs for any indications other than the specific conditions of use on the approved labeling. This extralabel use restriction precluded veterinarians from using an approved new animal drug for an unapproved animal species, for an unapproved indication, or for an approved species at dosage levels higher than that stated on the label. Prior to 1994, the use of human drugs for treating animals was also illegal.

In 1996, Title 21 of the CFR was amended to add part 530, entitled *Extra-label Drug Use in Animals*. This action served to implement AMDUCA to permit veterinarians to prescribe extralabel uses of certain approved animal and human drugs to treat animals under certain conditions. These regulations gave veterinarians the flexibility to meet patient needs in the practice of veterinary medicine.

Under AMDUCA, the key constraints are that any extralabel use must be by or on the order of a veterinarian within the context of a veterinarian–client–patient relationship; the use must not result in violative residues in food-producing animals; and certain listed compounds are prohibited from extralabel use in food-producing animals. The Agency may establish safe and violative levels for residues for extralabel use and may require development of analytical methods for residue detection. If, after affording an opportunity for public comment, FDA finds that an extralabel animal drug use presents a risk to public health or that no analytical method has been developed and submitted, the Agency may prohibit the extralabel use.

As of this writing, the Agency has prohibited the extralabel use of the following drugs in food-producing animals:

1) Chloramphenicol;
2) Clenbuterol;
3) Diethylstilbestrol (DES);
4) Dimetridazole;
5) Ipronidazole;
6) Other nitroimidazoles;
7) Furazolidone;
8) Nitrofurazone;
9) Sulfonamide drugs in lactating dairy cattle (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine);
10) Fluoroquinolones;
11) Glycopeptides;
12) Phenylbutazone in female dairy cattle 20 months of age or older;
13) Cephalosporins (not including cephapirin) in cattle, swine, chickens, or turkeys:
    a) for disease prevention purposes;
    b) at unapproved doses, frequencies, durations, or routes of administration; or
    c) if the drug is not approved for that species and production class.
14) The following drugs, or classes of drugs, that are approved for treating or preventing influenza A, are prohibited from extralabel use in chickens, turkeys, and ducks:
    a) Adamantanes
    b) Neuraminidase inhibitors.

Neither AMDUCA nor the implementing regulations lessen the responsibility of the manufacturer, the veterinarian, and the food producer to prevent risks to public health from drug residues in animal-derived foods. Updates on issues pertaining to the Animal Medicinal Drug Use Clarification Act of 1994 and its implementation by CVM can be found at: www.fda.gov.

The Animal Drug Availability Act of 1996

In an effort to increase the number of animal drugs on the market and to reduce regulatory burdens on the animal health industry without undermining the safety of animal drug products, the Animal Drug Availability Act (ADAA) was signed into law on October, 1996. The current study requirements associated with NADAs reflect changes instituted by this Act. In brief, the ADAA provides for:

- An amendment to the definition of substantial evidence of effectiveness that provided CVM with greater flexibility to make case-specific scientific determinations regarding the number and types of adequate and well-controlled studies that will provide, in an efficient manner, substantial evidence that a new animal drug is effective.
- Greater direct interaction between animal drug sponsors and the FDA during the drug development process. The law requires a presubmission conference, held at the sponsor's discretion, to discuss the drug development at its earliest stages. The goal is for the FDA and the manufacturers to reach a common understanding regarding what data will be needed to establish safety and effectiveness, and what types of studies can be conducted to generate such data.
- The ability to market an animal drug with a range of acceptable/recommended doses on animal drug product labeling, rather than as one optimum dose. In addition, the law directs FDA to broaden the approval

process to make available more animal drugs to treat minor species.

- A new category of drugs, "Veterinary Feed Directive Drugs" (VFD), that allows approval and use of new animal drugs in animal feed, on a veterinarian's order, while incorporating safeguards to ensure the safe use of such drugs. This new category of drug is discussed in Section Classifying Prescription, Over-the-Counter and Veterinary Feed Directive Animal Drugs.

Premarket Evaluation of New Animal Drugs

In 1968, the US Congress passed the animal drug amendments to the Federal, Food, Drug, and Cosmetic Act. In doing so, Congress recognized the need for the use of animal drugs to meet therapeutic and production needs for animals. Congress intended to create a structure that would enable the approval of new animal drugs that met the legal standards of safety and effectiveness.

In order to approve and sustain a new animal drug for commercial use, four critical pillars must be met. First, an animal drug product must be *safe*, safe for the animal, safe for the humans consuming food derived from treated animals, safe for the user or the person administering the drug and for the environment. Second, an animal drug must be *effective* for its intended uses. These uses are those prescribed, recommended, or suggested in the labeling of the product. Third, the animal drug must be a *quality manufactured product***,** being the result of a validated manufacturing process conducted in accordance with current Good Manufacturing Practice regulations. Fourth, the product must be *properly labeled* to inform the user of the product not only how to use the product but also safety considerations, residue withdrawal procedures, and storage and handling procedures. When these four characteristics are met, an animal drug can be approved for marketing. Once in the market place, an animal drug is monitored to ensure that these characteristics are sustained.

From CVM's perspective, its public health mission is achieved when a safe, effective, quality manufactured, properly labeled new animal drug is put in the hands of the user. Animal owners and veterinarians have many options for products that they might choose to use in treating animals under their care. Some of these options include unapproved drug such as illegal drugs, counterfeit drugs, or illegally compounded drugs. Each of these options fails to meet the characteristics of an animal drug that protects public and animal health. CVM and animal drug sponsors share a mutual interest in putting approved animal drugs in the hands of the user. CVM has an important responsibility to ensure that only safe, effective, quality manufactured, and properly labeled new animal drugs are in the marketplace for use and unsafe and ineffective products are not present.

Animal drugs are used to meet therapeutic and production needs for animals for a large number of animal species and indications. Consider that in the United States there are, 9 billion chickens, 262 million turkeys, 97 million cattle, 62 million pigs, 6.2 million sheep, and millions of aquaculture animals, and over 280 million pets in the USA, including 88 million cats and 75 million dogs.

For companion animals, CVM wants to ensure and increase the level of high-quality medical care for companion animals as well as improve the quality of life through medical interventions. Pets in the United States are closely bonded to their human owners. As such, many pets have significant close interactions with their owners presenting ideal opportunities for disease transmission. Many therapeutic drugs for companion animals serve an important human public health purpose preventing the development of zoonotic diseases when they are used in pets. Many companion animals serve an important role in their relationship with humans in terms of the wellness of people being directly linked to the human–animal bond. Healthy pets mean healthier people.

For food-producing animals, CVM wants to ensure that the use of therapeutic and production animal drugs maintain the health and improve animal welfare. In addition, by improving animal health and production, these animal drug products work to increase the availability of an affordable, abundant, and wholesome food supply to meet the needs of a growing human population. One of CVM's challenges is to engage in the development and evaluation of new animal drugs, especially new innovative technologies, to meet the demand for increased safe, affordable, and abundant food production.

For the animal drug industry to produce an adequate and diverse supply of animal drugs to meet therapeutic and production needs for animals, the regulatory processes must be constructed to not only meet the statutory requirements but also to be efficient and conducive to the further development of new animal drugs. At first look, these statements may seem contradictory and, at times, do cause substantial discussions. However, they are independent characteristics. CVM must first and foremost ensure that new animal drugs are safe and effective. CVM must prevent the introduction of unsafe and ineffective drugs into the marketplace as well as remove those that exist in the marketplace. At the same time, CVM must have efficient processes that are responsive to its stakeholders needs. This includes timely review decisions, effective and timely communication, and effective project management.

Without a doubt, the development and marketing of a new animal drug in the United States is a complex and onerous undertaking. Meeting the rigorous standards for safety and effectiveness and sustaining a quality manufactured product is a challenge. Having an approved new animal drug ensures protection for animals, the public, and drug manufacturers. By having consistent and equitable standards, sponsors are assured that competitors will have to meet essentially the same regulatory standards. Moreover, by providing a seal of approval that distinguishes the approved product from unapproved, illegal, counterfeit, and illegally compounded competing animal drug products, consumers of animal drug products have the assurance of a quality, reliable product.

The process leading to the approval and marketing of a new animal drug product is, in some respects, similar to that associated with human pharmaceuticals. In fact, many animal drugs were initially developed for potential use in humans. However, veterinary pharmaceutical development creates complexities that are not faced by our human pharmaceutical development. Some of these issues are:

- the diversity in size, behavior, metabolic needs, and lifespan between animal species;
- species and breed differences in both pharmacokinetic and toxicity profiles;
- a wide spectrum of disease agents that produce different disease manifestations under different conditions;
- a range of husbandry practices, which include the array of settings in which animals are kept – ranging from animal companions in the home to large livestock operations;
- inability to communicate directly with the animal patient;
- public health concerns, including environmental safety, human food safety, and the potential influence of veterinary antimicrobial use on development of resistance in bacteria of concern to both veterinary and human medicine.

There are also differences in the use of drug products in food-producing animal species versus companion animal species. The focus of diagnostics, drug therapy, and monitoring of treatment response is on the individual companion animal. The goals of therapy are to prevent, treat, or manage a disease or to improve the quality of life for the individual animal. Some therapies may be continued throughout the life of the companion animal. For food-producing animals, the focus is generally on the health of an entire group (herd or flock) of animals instead of an individual animal. Although food-producing animals may be individually dosed using injectable or topical products to treat or control certain diseases, group dosing via medicated drinking water or feed is often used in order to minimize handling stress and to allow for a more efficient means of dosing a large number of animals during a disease outbreak. The goal in food animal medicine is to manage the health of the herd.

A further challenge to animal drug development is the low cost and narrow profit margin of veterinary pharmaceuticals. Based upon information contained on the Animal Health Institute (AHI) website (http://www.ahi.org/about-animal-medicines/industry-statistics/), it takes about 7 to 10 years and up to $100 million to develop a new major animal drug product. On the average, when it comes to animal drug products, it is estimated that on an international front, the expenditure is only about 1/40th of what is spent on human medicine. In this regard, within the US, the sales associated with animal pharmaceuticals were between $5 and 6 billion (2010 estimates). In contrast, the total value of US consumption of pharmaceutical drugs in 2009 was $300 billion (http://www.bls.gov/ppi/pharmpricescomparison.pdf). Based upon information on the PhRMa website, annual pharmaceutical expenditure over the years 2009–2011 was estimated to be approximately $50 billion. In 2012, the amount of sales reinvested into research and development was 20.7% (http://phrma.org/sites/default/files/pdf/PhRMA%20Profile%202013.pdf).

The limited opportunity for cost recovery forms a barrier to increasing the availability of new animal products. As a practical matter, the amount of data contained in a New Animal Drug Application (NADA) is substantially less than that submitted to support a New Drug Application (NDA) for human use. For example, the number of patients enrolled in clinical trials of new animal drugs is substantially smaller than the thousands of patients generally enrolled in trials submitted in support of human NDAs.

Despite these constraints, the same statutory requirements regarding the demonstration of product safety and effectiveness apply to the regulation of both human and veterinary drug products. Therefore, innovative application of the principles of pharmacology and research study design is important in meeting the challenge of developing cost-effective ways to generate the data necessary to meet the statutory requirements of the FFDCA. Pharmaceutical companies are encouraged to consult closely with CVM to develop study designs and assays that maximize the value of the collected data to support their drug applications.

Components of New Animal Drug Applications

The legal requirements for NADAs are detailed in 21 CFR §514.1. Required information for evaluation of the NADA is grouped into technical sections and includes:

- Target Animal Safety;
- Effectiveness;
- Human Food Safety;
- Chemistry, Manufacturing, and Controls;
- Environmental Impact;

- All Other Information;
- Labeling.

The Office of New Animal Drug Evaluation (ONADE) within CVM is responsible for the review of information submitted by drug sponsors to obtain approval to manufacture and market animal drugs. Highlights for each of these technical sections are provided in the next several sections of this chapter.

Target Animal Safety

The purpose of the Target Animal Safety (TAS) studies is to evaluate potential harmful effects associated with use of a new animal drug in a controlled setting, as a prediction of product safety in the larger patient population. The regulation for TAS, 21 CFR §514.1(b)(8)(i), requires "adequate tests by all methods reasonably applicable to show whether or not the new animal drug is safe and effective for use as suggested in the proposed labeling". The target animal safety section may include studies that identify the toxic syndrome(s) associated with the drug and the margin of safety for use of the product in the treated animal. The FDA has guidance documents to summarize recommendations and suggestions for efficiently carrying out these studies.

As with human pharmaceuticals, published literature, preliminary studies (including pharmacokinetics and pharmacodynamics, and extrapolation from toxicity testing in laboratory animals), and safety observations in clinical effectiveness trials are considered in the safety evaluation. But for new animal drugs, safety is also directly assessed in the target species through laboratory-based (overdose) studies. These nonclinical laboratory studies must be conducted in accordance with Good Laboratory Practices (GLP) regulations (21 CFR Part 58). FDA does not require that domestic animals be maintained under the conditions required for laboratory animals during GLP testing. Although clinical effectiveness studies contribute data to the overall safety assessment of a drug, GLP regulations do not apply to clinical studies. Furthermore, the product evaluated in the TAS studies should be the same as the product intended to be marketed, that is the same chemical, same particle size, and formulation, and manufactured in conformity with the principles of Good Manufacturing Practices (GMP).

Although the specific information needed to assess TAS in a particular NADA depends on a variety of factors (e.g., proposed usage regimen and dose, type of drug, pharmacokinetics, chemistry and manufacturing considerations, claims, previous use history, and animal species including class and breed), the underlying scientific approach, as outlined in FDA guidance documents, is common to all. Studies generally compare healthy control animals with small numbers of healthy animals of the target species treated with multiples of both the recommended dose and duration of administration of the product. Appropriate observations, physical examinations, clinical pathology tests (hematology, blood chemistry, urinalysis, fecal analysis, etc.), necropsy, and histopathology should be conducted to identify possible adverse effects. Additional specialized studies, such as evaluation of injection sites, topical administration sites, reproductive safety and mammary gland safety, may be needed depending on the conditions of use and the characteristics of the drug. There may also be additional species-specific study requirements based upon unique physiologies and or husbandry practices. For example in fish, target animal safety can change as a function of water temperature, ionic strength and composition, and life stage (Storey, 2005).

Effectiveness

The availability of certain approved new animal drugs for use in livestock, poultry, pets, and other animals is vital to protecting the health of animals and the health of humans who consume the products of food-producing animals. The availability of other approved new animal drugs is vital to increasing the efficiency of food production in the United States. The changes made to the definition of "substantial evidence" by the ADAA and by the further definition of that term provided CVM with greater flexibility to make case-specific scientific determinations regarding the number and types of adequate and well-controlled studies that will provide, in an efficient manner, substantial evidence that a new animal drug is effective.

One or more adequate and well-controlled studies are required to establish, by substantial evidence, that a new animal drug is effective. These studies are intended to demonstrate that a new animal drug is effective for each proposed intended use and the associated conditions of use. The intended use includes the dose or dose range, frequency, duration, timing (e.g., in relation to the onset of clinical signs), animal species, age, gender, class, and breeds of animal for which the new animal drug is intended for use. Subsequent to the enactment of the ADAA, the number of adequate and well-controlled studies for providing substantial evidence of effectiveness depends upon the number of intended uses, how narrowly or broadly each intended use is defined, and upon the conditions of use in the proposed labeling. When a proposed therapeutic indication is associated with a dose range, substantial evidence of effectiveness is demonstrated for the lowest proposed dose. Sponsors also need to justify the intended dose. The basis of this justification can include pharmacokinetic/pharmacodynamic relationships, literature, and laboratory tests.

The technical section also includes information to support selection of the dosage (called dosage characterization). The pharmaceutical sponsor of the new animal drug may provide a logical scientific rationale or use a variety of studies in developing an effectiveness technical section, including studies conducted in the target species, studies conducted in laboratory animals, field investigations, bioequivalence studies, and in vitro studies. Pharmacokinetic and pharmacodynamic studies may augment and further document the nature of the animal drug's effectiveness. Prior to submission of the major technical sections, or as part of the effectiveness technical section, the sponsor provides the basis for choosing the dosage (dose, duration, and frequency) to be used in effectiveness studies. The sponsor may derive dosage characterization from many sources, including dose titration studies, pilot studies, foreign studies, published literature, in vitro studies, interspecies dose extrapolation, and pharmacokinetic and pharmacodynamic studies. In the dosage characterization submission, the sponsor provides a succinct dosage characterization narrative which is included in the Freedom of Information (FOI) Summary for the product.

The effectiveness section must contain full reports of all studies that show whether or not the new animal drug is effective for its intended use (21 CFR § 514.1(b)(8)(i)). The sponsor must demonstrate by substantial evidence that the drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling. 21 CFR §514.4(b)(2) requires that a sponsor submit data generated from well-controlled studies of a new animal drug to distinguish the effect of the new animal drug from other influences, such as spontaneous change in the course of the disease, normal animal production performance, or biased observation. The data provided for substantial evidence of effectiveness must permit qualified experts to conclude that a new animal drug will have the effect it purports or is represented to have under the conditions of use suggested in the proposed labeling (21 CFR §514.4(b)(3)(i)(C)). Generation of these data represents a significant component of drug development time and cost such that the amount and nature of the evidence needed can be an important determinant of whether and when new animal drugs become available to the public.

The effectiveness studies are designed to test the product under actual conditions of use. In contrast to studies conducted under controlled laboratory settings, the clinical trial provides information pertaining to how the product will perform under real world conditions in the patient population. The effectiveness studies are typically designed with a negative (placebo) control or an active control. An active control is an approved product of established effectiveness for the same indications as the proposed product under development, with a known margin of superiority relative to a placebo (Piaggio et al., 2006). The evaluation of the active control study should explain why the new animal drugs should be considered effective in the study, for example, by reference to results in previous placebo-controlled studies of the active control. The study is typically masked, and patients are randomized to treatment groups. In so doing, the investigator can evaluate the drug effects with minimal bias. The study typically follows a multicenter and multiinvestigator design to increase the likelihood that the product will perform as expected in the broader population postapproval. The study is testing whether the product will perform in the hands of multiple investigators under a variety of husbandry conditions, clinical practice settings, across different breeds and ages of the patient populations, and perhaps different stages of the disease or condition. The proposed indication drives the design of the effectiveness study. Likewise, the results of the effectiveness study drive the wording of the indication. The final approved indication and dosage are confirmed by the results of the effectiveness studies. Lastly, the effectiveness studies also provide important safety information about the use of the product in the patient population.

The manufacturing characteristics of the product used in the efficacy study must be provided as part of the study results in order to provide CVM with an assurance that the test article used is representative of the product intended for marketing.

Human Food Safety

The human food safety section is submitted only for applications for new animal drugs intended for use in species that are used for human food (food-producing animals). This section must include a description of practicable methods for determining the quantity, if any, of the new animal drug in or on food, and any substance formed in or on food because of its use, and the proposed tolerance or withdrawal period or other use restrictions to ensure that the proposed use of the drug will be safe (21 CFR § 514.1(b)(7)). This section should also contain any data relating to residue toxicology (including the impact of residues on human intestinal microflora), residue chemistry, and, if the new animal drug has anti-infective properties, microbial food safety.

The human food safety section may include, but is not limited to, short and long-term toxicology studies, total residue and metabolism studies, analytical method validation studies, tissue residue depletion studies, and microbial food safety information.

By the general safety provisions of Sections 409, 512, and 706 of the Federal Food, Drug, and Cosmetic Act, FDA must determine for each food additive, new animal drug, or color additive proposed for use in food-producing animals whether the edible products derived from treated animals are safe for human consumption. Accordingly, the sponsor must demonstrate *reasonable certainty of no harm* to human health for all drug products intended for use in food-producing animals.

A human food safety evaluation focuses on residues that are present in the intended type of food animal following intended use of the drug. A residue is "any compound present in the edible tissues of the target animal which results from the use of the sponsored compound, including the sponsored compound, its metabolites, and any other substances formed in or on food because of the sponsored compound's use" (21 CFR 500.82)

Details regarding human food safety assessments are provided in CVM Guidance #3 *General Principles for Evaluating the Safety of Compounds Used in Food-Producing Animals.*

The information needed to demonstrate human food safety for a NADA generally includes:

Evaluation of carcinogenicity: To establish the safety of veterinary drug residues in human food, a number of toxicological evaluations are recommended, including an assessment of the potential to induce neoplasia. Exposure to residues of veterinary drugs will usually occur at extremely low levels, but for potentially long periods. To ensure that substances that could pose carcinogenic potential at relevant exposure levels are adequately assessed, a number of issues should be considered, including genotoxicity, metabolic fate, species differences, and cellular changes. As part of the human food safety package, sponsors must determine whether or not carcinogenicity testing should be conducted. The decision to undertake carcinogenicity testing should take into consideration: (i) the results of genotoxicity tests, (ii) structure–activity relationships, and (iii) findings in systemic toxicity tests that may be relevant to neoplasia in longer-term studies. It should also take into consideration any known species specificity of the mechanism of toxicity. Any differences in metabolism between the test species, target animal species, and human beings should be taken into consideration.

Toxicological assessment: Toxicology tests are conducted in laboratory animals to determine the dose at which the compound produces an adverse effect and a dose that produces a no observed effect level (NOEL).

Determination of an Acceptable Daily Intake (ADI): The ADI is derived from the results of the study illustrating the most sensitive endpoint in the most appropriate species. The ADI is the highest dose used in the study that demonstrates a NOEL divided by an appropriate safety factor. As a general rule, CVM will use the safety factors indicated below in this section for the various types of studies.

The ADI is estimated as:

$$\text{ADI } (\mu\text{g/kg/day}) = [(\text{NOEL } (\mu\text{g/kg}))/ (\text{Safety Factor } (\text{day}^{-1}))]$$

Table 55.2 Safety factors associated with the different kinds of toxicology investigations

Type of Study	Safety factor
Chronic	100
Reproduction/teratology	(100 or 1000; 100 for a clear indication of maternal toxicity, 1000 for other effects)
90-Day	1000

The appropriate safety factor depends upon the type of study being used for establishing the ADI, as described in Table 55.2.

Determination of a safe concentration: The safe concentration is, by definition, the amount of residue that can be eaten in any edible tissue each day for an entire lifetime without exposing the consumer to residues in excess of the ADI. To estimate the safe concentration, FDA considers the ADI, the weight in kg of an average adult human (60 kg), and the amount of the product that may be consumed in grams per day (where consumption values, in grams consumed per day, are 300 for muscle, 100 for liver, 50 for kidney, and 50 for fat):

$$\text{safe concentration } (\mu\text{g/g}) = \frac{\text{ADI } (\mu\text{g/kg/day}) \times 60 \text{ kg}}{\text{grams consumed per day}}$$

Once a safe concentration has been determined, methods are developed and validated that will allow future testing of animal tissues to monitor for residues. Withdrawal times are determined to allow use of the drug with any residues expected to be below the safe concentration.

Selection of a marker residue: The marker residue is a residue used to monitor the depletion of total residues in edible tissues in a food animal. The marker residue must have a demonstrated relationship with the total radiolabel drug residues such that when the concentration of the marker residue is less than a specific value (the tolerance) in the target tissue, the total residues in all of the edible tissues are less than their respective safe concentrations.

Development and validation of an analytical method to measure the marker residue: This method serves as the regulatory method for determining the withdrawal time and for assessing whether or not animal-derived products contain residue concentrations in excess of the established limit (violative residues of meats and animal-derived food products). This method will be used after a drug is approved by validated analytical laboratories during inspections, insuring the absence of violative residues in marketed human food products derived from animal sources.

Establishment of a tolerance: A tolerance (the FDA maximum concentration of the marker residues within the target tissue) or the European version of a tolerance, the maximum residue limit (MRL) is determined based on the marker residue. The target tissue for the tolerance is generally the edible tissue from which residues most slowly deplete.

Establishment of a withdrawal time: The withdrawal time is the time between the last administered dose and when the drug residues drop below the safe concentration. The regulatory objective of the FDA is to predict a time when we can be 95% certain that the tissue residue concentrations in 99% of the animal population receiving the drug product (when dosed in accordance to the approved product label) will be at or below tolerance. Generally, multiple animals are sampled across at least four time points and regression methods with tolerance limits are used to set the withdrawal time. Concentrations of the marker residue must be measured with the same FDA-approved analytical method that will be used for the regulatory inspections. These topics are also discussed in Chapter 61 of this text.

For antibacterial drugs, additional information is assessed as part of the human food safety section. Two types of potential effects of the new animal drug on bacteria of human health concern are considered (see Section Microbiological Effects on Bacteria of Human Health Concern).

Microbiological Effects on Bacteria of Human Health Concern: The level of antimicrobial residues present in food should not have clinically relevant effects on the human intestinal microflora. Consideration is given to potential microbiological effects on food-borne bacteria of human health concern (FDA/CVM Guidance for Industry #144, Pre-Approval Information for Registration of New Veterinary Medicinal Products for Food-Producing Animals with Respect to Antimicrobial Resistance VICH GL27 and FDA/CVM Guidance for Industry #159 Studies to Evaluate the Safety of Residues of Veterinary Drugs in Human Food: General Approach to establish a Microbiological ADI VICH GL36R). FDA/CVM Guidance for Industry #152 Evaluating the Safety of Antimicrobial New Animal Drugs with Regard to Their Microbiological Effects on Bacteria of Human Health Concern provides guidance for characterization of antimicrobial resistance selection in bacteria of human health concern associated with the use of antimicrobials in food-producing animals. FDA/CVM then uses the resulting risk estimation ranking, along with other data and information submitted in support of the NADA, to determine if the drug is approvable under specific risk management conditions.

Chemistry, Manufacturing, and Controls

The Chemistry, Manufacturing, and Controls technical section is one of the most important as it establishes the identity, strength, and purity of the product as well as the safeguards, unique to approved drugs, that the product will remain the same batch to batch when in commercial production. This enables the user of the product to be able to rely on the data generated in the development of the product to continue to represent the established safety and effectiveness of the marketed product. Several pivotal considerations go into the evaluation of the chemistry and manufacturing of a new animal drug product. These considerations are intended to ensure the quality and performance of the product, both prior to and subsequent to marketing. CVM requires that sponsors provide the information needed to ensure that each lot of the product released to consumers performs in a consistent manner, and that consumers have the necessary information with regard to product storage conditions so that product quality and performance are maintained (21 CFR §514.1).

A full description of the methods used in, and the facilities and controls used for, the manufacture, processing, and packaging of the new animal drug must be provided in the new animal drug application. This description should include full information with respect to any new animal drug in sufficient detail to permit an evaluation of the adequacy of the described methods of manufacture, processing, and packing, and the described facilities and controls. This information is evaluated by CVM's Division of Manufacturing Technologies to determine the identity, strength, quality, and purity of the new animal drug. Information is also needed with regard to the methods used in the synthesis, extraction, isolation, or purification of any new animal drug and the precautions being taken to ensure proper identity, strength, quality, and purity of the raw materials, whether active or not. Sponsors must provide CVM with the instructions used in the manufacturing, processing, packaging, and labeling of each dosage form of the new animal drug, and precautions being taken to ensure batch-to-batch product uniformity.

To ensure adequate performance as the product ages, the sponsor also must conduct studies of the stability of the new animal drug in the final dosage form. Expiry dates represent the duration of time for which product quality and performance can be assured if the product is stored in accordance with label recommendations. The time for expiry is based upon extensive stability testing that is conducted by the drug sponsor on the product intended for marketing, and these data are evaluated by the Division of Manufacturing Technologies at CVM.

Environmental Impact

The environmental impact section must contain either an environmental assessment (EA) under 21 CFR § 25.40, or a request for categorical exclusion under 21 CFR § 25.33 (21 CFR § 514.1(b)(14)). Environmental information is submitted to comply with the National

Environmental Policy Act (NEPA). Before approving a new animal drug, the agency must consider potential effects on the environment. In many cases, including those for many intended uses for minor species, a categorical exclusion from the need to provide an EA can be granted. In other cases, such as new chemical entities, and almost always in the case of a drug for an aquatic species approval, some type of EA will be necessary to support a “finding of no significant impact” (FONSI). The environmental assessment may include information on the introduction of the drug into the environment through manufacture, use, and disposal, the fate of the drug in the environment, and the effects of the drug in the environment.

Environmental documentation addresses the potential impact of the manufacturing and use of the product if the application were approved. The FDA Environmental Assessment Technical Handbook is available through the National Technical Information Service to assist in determining the contents of environmental documents. GLP compliance is required for all environmental laboratory studies. Preapproval GMP inspections are used to confirm the contents of environmental documents that apply to manufacturing environmental permits and controls.

The overall target of the assessment is the protection of ecosystems. The field of ecotoxicology is a complex science and gaps in data and knowledge exist. Nevertheless, CVM and the International Veterinary Cooperative and Harmonization (VICH) have developed guidance (CVM Guidance #166 dated 1/09/2006) that describes the kinds of information needed as part of the Environmental Impact Assessment (EIA) of veterinary medicinal products (VMPs).

The goal of the EIA is to assess the potential for VMPs to affect nontarget species in the environment, including both aquatic and terrestrial species. It is not possible to evaluate the effects of VMPs on every species in the environment that may be exposed to the VMP following its administration to the target species. The taxonomic levels tested are intended to serve as surrogates or indicators for the range of species present in the environment. Impacts of greatest potential concern are usually those at community and ecosystem function levels, with the aim being to protect most species. However, it may be important to distinguish between local and landscape effects. There may be some instances where the impact of a VMP at a single location may be of significant concern, for example, for endangered species or a species with key ecosystem functions. These issues are handled by risk management at that specific location, which may even include restriction or prohibition of use of the product of concern in that specific local area. Additionally, issues associated with cumulative impact of some VMPs may be appropriate at a landscape level.

The route and quantity of a VMP entering the environment determines the risk assessment scenarios that are applicable and the extent of the risk assessment. The EIA is based on the accepted principle that risk is a function of the exposure, fate, and effects assessments of the VMP for the environmental compartments of concern. While emission can occur at various stages in the life cycle of the product, with the exception of certain topicals or those added directly to water, most VMPs first pass through the animal to which it is administered. Generally, the most significant environmental exposure results from excretion of the active substance(s).

All Other Information (AOI)

The All Other Information technical section includes all information pertinent to the review of safety and effectiveness received or otherwise obtained by the applicant from any source (see 21 CFR § 514.1(b)(8)(iv)). The AOI technical section is usually submitted to the CVM ONADE Target Animal Division when the last major technical section is submitted for review and contains information from any investigations, foreign marketing, and scientific literature, and any other data that were not already submitted by the sponsor as part of a major technical section.

This information should be comprehensive and balanced and include favorable and any unfavorable literature. Although this technical section is submitted towards the end of the drug approval process, the sponsor should also submit AOI with each technical section. For example, the effectiveness technical section would include not only dosage characterization and substantial evidence data but also any AOI available at that time. Then when the final AOI technical section is submitted, it would contain only new information identified since the Effectiveness technical section was considered complete.

Product Labeling

The ability to interpret and understand drug labels is essential in ensuring that use of a particular drug achieves the intended outcome and minimizes unintended effects. The product labeling is the avenue through which CVM communicates instructions about product use and storage conditions to veterinarians, as well as communicating the benefits and risks associated with the use of a drug product.

Section 201K of the FFDCA defines “label” as a display of written, printed, or graphic matter upon the immediate container of any article. A drug’s “labeling” includes all labels and other written, printed, or graphic matter, such as package inserts, on the drug, its containers or wrappers or accompanying the drug. Labeling information is derived from studies submitted by the pharmaceutical sponsor to the FDA to support new animal drug approval and is developed as a collaboration between FDA and the drug sponsor.

Throughout the product label, the FDA provides the practitioner with a thorough understanding of the benefits and risks associated with the use of a drug product in the animal patient. The concept of "safe use" includes safety to the animal patient, safety to the person administering the product, the safety of food derived from the treated animal, and environmental safety issues associated with use or disposal of the product. Safety information on the labeling can be derived from studies conducted by the pharmaceutical sponsor, studies from peer reviewed scientific literature, or from extrapolation from human drug labeling information that reasonably applies to the target species.

Safety information is provided throughout the package insert under several different headings, including Contraindications, Warnings, Precautions, Adverse Reactions, and Animal Safety. The labeling is evidenced-based information, and does not represent opinions or conjecture. Therefore, for the practitioner to fully understand how the animal drug will perform, the practitioner should become familiar with all sections of the package insert to better understand both the risks and benefits associated with use of the product. The safety information can be viewed from the perspective of a hierarchy of severity, with Contraindications being the most serious, followed by Warnings and Precautions. The Animal Safety section is generally derived directly from laboratory target animal safety studies. The Adverse Reactions section is developed directly from the preapproval field effectiveness studies and the postapproval experience after the drug is marketed to the larger population. The Contraindications, Warnings, and Precautions statements can be modified by new findings from the postapproval surveillance of product use.

Typical sections/information found on a package insert includes:

- **Product Identification, Description and Prescription Legend** provides the product's brand name (often indicated by "®"), the generic name (the name given to the chemical entity), the dosage form (e.g., tablet, sterile solution, oral suspension, etc.), and if designated as a prescription or VFD drug, the prescription or VFD legend. The labeling for all veterinary prescription products contains the following statement: "Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian." The prescription (Rx), over-the-counter (OTC), and VFD classification is discussed later in this chapter.
- **The Indications section** of the labeling lists the specific disease(s) or conditions(s) for which a particular drug product is approved. The specific wording in this section depends upon the data contained in the effectiveness technical section of the approved new animal drug application.
- **The Contraindications section** defines the known circumstances under which a product should not be used because the risk associated with drug use clearly outweighs the possible benefit. Contraindications are based upon reasonable evidence that a drug caused a specific adverse reaction. Use of the drug in the situations described in the Contraindications section is likely to result in a serious adverse event that is fatal, or life-threatening, or requires professional intervention, or causes an abortion, or stillbirth, or infertility, or congenital anomaly, or prolonged or permanent disability or disfigurement to the treated animal.
- **The Warnings section describes** serious adverse reactions and potential safety hazards to the animal being treated. Although the adverse reactions may be serious, FDA has determined that the benefit of using the drug outweighs the risk of the adverse event.

The Warnings section may also describe potential actions that could be taken by the veterinarian to mitigate the adverse reaction should it occur, or to decrease the likelihood of the adverse reaction occurring, such as avoiding use in a predefined high-risk population. Warning statements are based upon evidence indicating an association of a serious hazard with a drug, but not necessarily proof that the drug caused the reaction. Significant problems that may lead to death or serious injury may require a "boxed warning" on the package insert.

The Warnings section may also include a user safety (Human Warnings) or human food safety section. The user safety section provides information regarding hazards to human health by contact, inhalation, ingestion, injection, or by other exposure to the product. The human food safety (Residue Warnings) section states the withdrawal time for use of the product in a food animal species. The withdrawal time is the time interval between the last administration of the drug to the food animal and the time when the animal can be slaughtered, or products, such as milk or eggs, can be used for food, without incurring violative drug residues in the food product. This section also warns against use of the drug in animals for which a withdrawal time has not been determined.

- The **Precautions** section includes additional information to enhance the safe use of the product by the practitioner. The Precautions section includes any additional information that is important for the safe use of the product that has not been included in the Contraindications or Warnings sections. The Precautions section may include recommendations for screening or diagnostic tests, information about drug to drug interactions, carcinogenesis, reproductive safety, adverse reactions in species other than the target species, or use in particular subpopulations of the target species, such as pediatric, geriatric, or animals with a particular disease.

- The **Adverse Reactions** section of the labeling lists undesirable effects reasonably associated with the use of the drug that may occur as part of the pharmacological action of the drug or that may be unpredictable in their occurrence. The Adverse Reactions section is developed from the field effectiveness studies conducted by the pharmaceutical sponsor to support new animal drug approval. This section may also include a postapproval experience section, reporting any additional undesirable effects reported from the postapproval surveillance of the product in the larger population. The Adverse Reactions section includes a toll-free number for the practitioner to call for technical assistance from the pharmaceutical sponsor.
- The **Information for Owner or Person Treating Animal** section contains specific information for the owner or person treating the animal regarding the safe and effective use of the product. This information is designed to be conveyed by the prescribing veterinarian to the animal owner. Some products have a separate client information sheet (CIS) in addition to the package insert. The CIS sheet is designed to be given to the animal owner every time the product is dispensed. The CIS is typically required by the FDA when there is a need to give the animal owner detailed information about the potential for serious adverse reactions from the drug. The CIS may include information to help the animal owner recognize and report to the veterinarian any early signs of problems following the use of the product in the individual patient.
- The **Clinical Pharmacology** section is a concise summary of the pharmacology, pharmacokinetics, and pharmacodynamics of the drug in the target species. This section may also provide information about the drug class, potential drug–drug interactions, and the effect of food on product bioavailability. For an antimicrobial agent, this section often includes a microbiology section with susceptibility data for the specific pathogens for which the product is approved.
- The **Effectiveness** section of the package insert contains an abstract of the results from the effectiveness studies published in the FOI Summary. The abstract provides a concise summary of the pivotal effectiveness studies and includes information such as the number and geographic location of study sites, the age, sex, and total number, etc. of animals in the study, a brief description of the study design, and the study results. The abstract describes how the effectiveness studies were conducted and how well the treated animals fared as compared to the control animals. This information can be considered from the perspective of the similarity between the study population versus the potential patient undergoing treatment (where the "patient" can represent an individual animal or a group of animals). For example, relevant questions may include whether or not the patient and the study population are of a similar age, class, and level of morbidity. This kind of information may be particularly useful when choosing between similar products that are approved and available for the same indications.

With regard to the database supporting product approvals, more detailed information than provided by the abstract can be obtained in the FOI summary. These summaries are easily accessed from CVM's website by simply inserting the NADA or ANADA number (another piece of information found on the package insert) for that product.

- The **Animal Safety** section provides an abstract of the results from the TAS studies that are required for FDA approval of the new animal drug. This information demonstrates the margin of safety for the drug. It also allows the reader to see what types of signs were observed in animals that received the drug at higher doses and for longer times than the intended dosage. It should help the practitioner decide whether or not to use the drug in animals that may be old, young, or have compromised organ function. If adverse signs in a treated animal are observed, the package insert provides information regarding whether or not similar signs were noted during the safety testing of this application.
- The **Storage Information** section describes the recommended storage conditions that are needed to maintain the potency of the drug within acceptable limits before the established expiration date. When applicable, this includes practical information for shipping, warehouse storage, and storage by the user. Any mandatory storage conditions are described (e.g., Store at controlled room temperature, 20–25°C (68–77°F)). When the drug is to be mixed with a diluent before use, this section describes the storage conditions for the diluted drug and also the time limitations for use of the diluted drug.
- **How the Product is Supplied** can include a description of the delivery device, the number of units per package, and the dosage strengths.
- The **NADA or ANADA Number and FDA Approval Statement:** The NADA or ANADA number can be used for searching the FDA/CVM website or for reporting product-specific adverse reactions. The presence of this section ensures the practitioner that they are using an approved new animal drug.

Classifying Prescription, Over-the-Counter and Veterinary Feed Directive Animal Drugs

FDA is responsible for determining the marketing status (Rx, OTC, or VFD) of animal drug products based on whether or not it is possible to prepare "adequate directions for use" under which a layperson can use the drugs safely and effectively. Prescription (Rx) products can be dispensed only by or upon the lawful written order of a licensed veterinarian.

Considerations include the potential toxicity or other harmful effects of the product, its method of administration, collateral measures necessary for its use (e.g., accurate diagnosis of a disease with reasonable certainty), and that the course of therapy can be followed for other potential safety risks or lack of success of the product.

The same drug substances can be marketed in a number of different dosage forms, intended for use by different routes of administration, and in different species of animals. Thus, these drug products may be appropriately labeled Rx in some cases and OTC in others. Veterinary prescription drugs must bear the legend: "Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian."

Before the passage of the ADAA, the FFDCA provided FDA only two options for regulating the distribution of animal drugs: OTC and Rx. The prescription legend invoked the application of State pharmacy laws, and the pharmacy laws in a significant number of States prohibited feed manufacturers from possessing and dispensing prescription animal drugs and medicated feed containing those drugs. Pharmacy laws in other States required the presence of a pharmacist at the feed manufacturing facility that uses prescription drugs in the manufacture of medicated feeds. As a practical matter, the application of State pharmacy laws to medicated feeds would burden State pharmacy boards and impose costs on animal feed manufacturers to such an extent that it would be impractical to make these critically needed new animal drugs available for animal therapy. For this reason, through the ADAA, Congress enacted legislation to create the VFD, providing a new class of restricted feed use drugs that may be distributed without invoking State pharmacy laws. Although statutory controls on the distribution and use of VFD drugs are similar to those for prescription animal drugs regulated under section 503(f) of the FFDCA (21 U.S.C. 353(f)), the VFD regulations are tailored to the unique circumstances relating to the distribution of animal feeds containing a VFD drug. Unlike prescription drugs, VFD drugs are not regulated by State pharmacy bodies. No extralabel use of a VFD drug is permitted and a veterinarian may issue a VFD only if a valid veterinarian–client–patient relationship exists, as defined in 21 CFR §530.11(b).

Abbreviated New Animal Drug Applications

Prior to GADPTRA, the FDA could approve abbreviated applications only for copies of animal drugs approved prior to 1962 and found to be effective under the DESI (drug efficacy study implementation) program. The intent of GADPTRA was to encourage competition and lower animal drug prices by allowing abbreviated applications for copies of previously approved drugs, without the generic drug sponsor duplicating the safety and efficacy studies that were required for the original NADA approval. GADPTRA permits an ANADA for an animal drug product that is the same as an animal drug product listed in the approved animal drug product list published by the Agency (listed new animal drug) with respect to conditions of use recommended in the product labeling, active ingredient(s), dosage form, strength, and route of administration.

An ANADA applicant may petition the Agency under Section 512(n)(3) of the FFDCA for permission to file an ANADA for a new animal drug product with certain defined changes from an approved pioneer product. The changes are limited to the following:

- a change of one active ingredient in an approved combination product;
- a change in dosage form;
- a change in dosage strength;
- a change in route of administration;
- a change in one active ingredient in a feed mixed combination.

A generic sponsor may file a suitability petition to request that the specific change in the pioneer product be allowed. The petition must follow the format and content described for a Citizen Petition (21 CFR §10.20) which is a public document filed with the FDA. The FDA response to the petition is also a public document. The Agency determines whether to deny or approve the petition.

ANADAs need to address all of the technical sections applicable to NADAs, but, in some cases, the information needed to fulfill these requirements is different. For example, target animal safety and effectiveness are evaluated through the demonstration of product bioequivalence between the pioneer and generic products. The focus of bioequivalence studies is to determine whether or not differences in product manufacturing and formulation will affect the rate and extent of drug absorption. The fundamental assumption of all bioequivalence testing is if the rate and extent of drug absorption are comparable, then the products will be medically indistinguishable and therefore interchangeable.

Bioequivalence studies (i.e., blood level, pharmacological end-point, and clinical end-point studies) and tissue residue depletion studies are conducted in accordance with GLP regulations (21 CFR Part 58). When absorption of the drug is sufficient to enable the quantification of drug concentrations in the blood (or other appropriate biological fluid or tissue) and if systemic absorption is relevant to drug action, a blood (or other biological fluid or tissue) level bioequivalence study should be conducted.

For certain generic drug products, the Agency may waive the requirement for demonstration of bioequivalence to the pioneer product. In general, the bioequivalence between solutions is considered to be self-evident for solutions with the same active and inactive ingredients in the same concentrations and with the same pH and physicochemical characteristics as the pioneer product. Categories of products that may be eligible for waivers include parenteral solutions intended for injection by the intravenous, subcutaneous, or intramuscular routes of administration, oral solutions or other solubilized forms, topically applied solutions intended to produce local therapeutic effects, and inhalant volatile anesthetic solutions.

When using serum or blood concentrations of drug in the evaluation of product bioequivalence, the principles underlying this assessment reflect a blending of pharmacokinetics and biostatistics. It is founded upon the principle that two formulations containing the identical active ingredient will exhibit the same safety and effectiveness if the two formulations exhibit the same rate and extent of absorption. In addition to its use in generic drug product applications, bioequivalence studies may be used to support the approval of proposed changes in the manufacturing of a generic version of an approved off-patent product or to cover the safety and/or effectiveness of a sponsor's own drug.

Blood level bioequivalence studies compare a test formulation to a reference formulation using parameters derived from the concentrations of the drug moiety and/or its metabolites (as a function of time) in the plasma, serum, or other appropriate biological fluid. This approach is applicable to dosage forms intended to deliver the active drug ingredient(s) to the site of action by way of the systemic circulation. Although usually conducted as a comparison of blood levels following single dose administration, a multiple dose study may be appropriate when there are concerns regarding poorly predictable drug accumulation. For details regarding the assessment of product bioequivalence, readers are referred to CVM Guidance #35 (*Bioequivalence Guidance*) as well as Chapter 3 of the present textbook.

Since veterinary generic drug products must contain all of the same indications, warnings, cautions, directions for use, etc. that are associated with the approved pioneer product (except as deemed appropriate on the basis of the Suitability Petition (21 CFR §512(n)(1)(F)), a bioequivalence study is generally needed for each species for which the pioneer product is approved (with the exception of "minor" species).

When the product in question is a generic product intended for use in a food-producing animal, sponsors are required to conduct both bioequivalence and tissue residue studies unless blood concentrations of the active drug can be measured out to the withdrawal time of the pioneer product. The requirement is based upon the conclusion that tissue residue depletion of generic products is not adequately addressed through blood level bioequivalence studies. Differences that may go undetected in a blood level study (and which would be without clinical significance) could impact the withdrawal time because the latter is based upon extremely low drug concentrations in the tissue (see section Human Food Safety). A tissue residue study should generally accompany clinical end-point and pharmacological end-point bioequivalence studies (21 USC 360b(n)(1)(A)(ii)).

The sponsor of the generic product application uses the existing tolerance, marker residue, target tissue, and analytical method (as contained within the pioneer product's approved NADA) to determine the withdrawal time for the generic product (FDA/CVM Guidance For Industry #35). Accordingly, generic sponsors need only monitor the depletion of the unlabelled marker residue in the target tissue to establish a withdrawal time for the generic product. However, for reasons explained above, there is a risk that the generic formulation will be associated with a withdrawal time that differs (longer or shorter) than that of the pioneer product. For this reason, the end-users of generic products should carefully read each product label to avoid the potential for incurring violative residues.

The Phased Review Process

For a sponsor to market a new animal drug in the United States, the sponsor must obtain an approval for their product through an NADA. Traditionally, a sponsor would generate all of the necessary data and information and file a complete NADA. Because of the complexity of an NADA, the likelihood of each component of the NADA being acceptable without working closely with CVM is small. This means that the sponsor has probably spent a significant amount of effort and resources developing an application that will need to be rehabilitated. This is not only inefficient but is also costly. For this reason CVM developed a Phased Review process where a sponsor could work with CVM to develop the components or technical sections of their NADA prior to filing their NADA. This higher level of interaction enables the sponsor to have each piece of their application reviewed under the investigational phase and receive feedback on the acceptability of their submission. Sponsors may submit study protocols, final study reports, and other information for review as they are produced.

Presubmission Conference

The Phased Review process starts with a meeting or series of meetings known as the presubmission conference. This meeting is very important for the success of the product development. During this meeting the sponsor provides scientific and technical information about their product and the proposed intended use(s) for their product. The sponsor outlines the safety and effectiveness issues that they perceive need to be addressed and how they intend to address them. CVM discusses these development plans with the sponsor leading to an understanding of the items that must be addressed for a successful development plan.

After the presubmission conference, a sponsor will work on each of the parts of the application or technical sections. The technical sections are target animal safety, effectiveness, human food safety, manufacturing chemistry, environmental impact, labeling, and all other information.

Outcomes of this conference will be documented in a memorandum of conference (MOC) as specified in 21 CFR 514.5.

Study Protocols

Submissions

Sponsors are not required to submit study protocols for review but do so voluntarily. If CVM concurs with the submitted protocol CVM is agreeing that the protocol design, execution plans, and data analyses are adequate to achieve the objectives of the study. If CVM does not concur, a letter will be given to the sponsor with as much detail as possible considering the quality and level of detail of the protocol submission to include a succinct assessment of the protocol. This response will also state whether the Agency agrees, disagrees, or lacks sufficient information to reach a decision.

Protocols should be well-written, clear, concise, and consistent across all sections so the investigator has a document outlining the study methodology and procedures. Data collection forms (e.g., case report forms, owner consent forms) should be included with the protocol and referenced in the section of the protocol where they are discussed. Relevant standard operating procedures (SOPs) for laboratory studies related to the collection of data should be appended to the protocol or an adequate description of the procedure should be incorporated into the protocol (for example, SOPs for describing parasite collection for anthelmintic studies or SOPs for culture and sensitivity microbiological procedures). If unsure, the sponsor should contact CVM to discuss which, if any, SOPs should be included with the protocol. Lack of detail in a study protocol may result in a refusal to review by CVM.

Protocol Development Meetings

Sponsors can request the scheduling of a protocol development meeting to discuss the specific details of a protocol. CVM strongly encourages a protocol development meeting for novel indication(s), novel products, and complex study designs. When requesting a protocol development meeting, sponsors should consider the timing of the meeting in relation to the submission of the protocol and any additional supportive information that may be needed.

Study Documentation and Submission

Using the protocol, a sponsor will conduct the study. Effectiveness studies are conducted in accordance with GCP Guidance, and safety studies are conducted in accordance with GLP regulations. When the study is completed and the final study report written, the sponsor will provide CVM with a study submission that contains the final study report and supporting documentation including the raw data for review. CVM will review the study for data integrity and for scientific merit. If the study is accepted, it will be used to support either a portion of or the complete technical section. When the entire technical section is complete, CVM will send the sponsor a technical section complete letter.

Administrative New Animal Drug Application

When a sponsor has received technical section complete letters for each of the required technical sections submitted to support approval of a new animal drug, the sponsor may file an Administrative NADA. The Administrative NADA contains a number for critical documents including the complete facsimile labeling, copies of all the technical section complete letters, and a signed FDA Form 356V. Upon receipt of an Administrative NADA, CVM will review and process the application to approval within 60 days.

Animal Drugs Intended for Minor Use/Minor Species

The Minor Use and Minor Species Animal Health Act of 2004 (the MUMS Act) was enacted to increase the availability of new animal drugs for minor species and for minor uses, while still ensuring appropriate safeguards for animal and human health. The MUMS Act provided for the establishment of the Office of Minor Use and Minor Species Animal Drug Development (OMUMS). OMUMS is responsible for overseeing the development and legal marketing of new animal drugs for minor uses (disease conditions that are rare) in major species (cattle, swine, chickens, turkeys, dogs, cats, and horses) and for minor species that fall under the category of "all other animals", including sheep, goats, game birds, emus, ranched deer, alpacas, llamas, deer, elk, rabbits, guinea pigs, pet birds, reptiles, ornamental and other fish, shellfish, wildlife, zoo animals, aquarium animals, and bees.

Because the markets are small and profit margins low for new animal drugs intended for minor uses in major species or for minor species, there are often insufficient economic incentives to motivate sponsors to develop the data necessary to support FDA approvals. In addition, some minor species populations are too small or their management systems too diverse to make it practical to conduct traditional studies to demonstrate safety and effectiveness. Consequently, manufacturers have not, in many cases, been willing to fund research to collect these data. Accordingly, very few new animal drugs intended for minor uses or for minor species have been approved and are legally marketed.

The limited availability of approved new animal drugs intended for minor uses or minor species, has limited the availability of options for treating these sick animals. In many cases, the choices are to leave a sick animal untreated or to treat the animal with an unapproved drug. Failure to treat sick animals appropriately may increase public health hazards. For example, the transmission of disease from animals to humans or the shedding of disease-producing organisms by untreated animals into the environment may increase health risks to humans as well as other animals. Treating an animal with an unapproved drug introduces questions of effectiveness and safety to the animal, to the environment, and to the public (e.g., human food safety).

The MUMS legislation modifies provisions of the FFDCA in three key ways, providing for:

- **Conditional Approval**: The sponsor of a veterinary drug can ask CVM for "conditional approval," which allows the sponsor to market the drug after proving the drug is safe and establishing a reasonable expectation of effectiveness, but before collecting all of the effectiveness data needed to support a full approval. The drug sponsor can keep the product on the market for up to 5 years, through annual reviews, while gathering the required effectiveness data.
- **Indexing:** The implementation of this regulation allows the FDA to add a minor species drug to an index of unapproved new animal drugs that may be legally marketed when the potential market for the drug is too small to support the costs of the drug approval process, even under a conditional approval. This provision is especially helpful to veterinarians treating zoo or endangered animals, and to owners of minor pet species such as ornamental fish or caged reptiles, birds, or mammals.
- **Designation**: This legislation provides incentives for approvals, such as grants to support safety and effectiveness testing. Sponsors must apply for designation prior to filing a new animal drug application for FDA approval. At the time that a designated drug gains approval or conditional approval, it is awarded 7 years of exclusive marketing rights. This means that FDA will not approve another application for the same drug in the same dosage form for the same intended use until after the 7 years have elapsed. This is 2–4 years longer than the protection provided from generic copying of nondesignated drugs. The MUMS marketing exclusivity also protects against approval of another pioneer (nongeneric) application for the same drug, in the same dosage form, for the same intended use.

Grants: The legislation also includes a provision that will allow Congress to appropriate funds for grants to defray the costs of safety and effectiveness testing for designated drugs.

Postapproval Monitoring of Animal Drugs

Postapproval monitoring of animal drugs is a very important function, necessary to ensure that the approved new animal drugs continue to be manufactured in accordance with their approval specifications and continue to perform as expected. In addition, CVM monitors the advertising and promotional material used by pharmaceutical sponsors to ensure that the product is represented in accordance with the conditions of its approved application. CVM uses Drug Experiencing Reports (DERs), Adverse Drug Experience Reports, and continued manufacturing facility inspections to conduct this monitoring.

Drug Experience Reports

Sponsors holding approved new animal drug applications are responsible for establishing and maintaining records concerning experience with the drug and Type A Medicated Articles containing the drug and for making reports of those experiences (21 CFR 514.80). Sponsors must file DERs within 30 days of the reporting period. After the initial approval, the first two reporting periods are at 6-month intervals, after which the reports are to be filed on an annual basis on the anniversary date of the approval.

The DER submission includes:

- unpublished reports of clinical or other animal experience, studies, investigations or tests conducted or reported to the applicant;
- experience, investigations, or studies involving the physical or chemical properties of the animal drug;

- copies of labeling that accompany the drug and copies of promotional labeling;
- advertising for drugs that are labeled for use by or on the order of a licensed veterinarian;
- quantities of the drug distributed to facilitate assessment of adverse effects;
- summary of reports of increased frequency of adverse drug experience (ADE)(See Section Adverse Drug Experience Reports);
- mix-ups with the new animal drug or its labeling;
- changes or deterioration of the new animal drug or failure of a batch to meet specifications;
- unexpected side effects, injury, toxicity, sensitivity reaction, or unexpected incidence or severity of side effects associated with chemical use irrespective of attribution to the new animal drug;
- failure of new animal drug to exhibit expected pharmacological action.

Adverse Drug Experience Reports

The primary purpose for maintaining the FDA/CVM ADE database is to provide an early warning or signaling system for adverse effects not detected during premarket testing of FDA-approved animal drugs and for monitoring the performance of drugs not approved for use in animals. The FDA/CVM ADE reporting system depends upon the detection of an adverse clinical event by veterinarians and animal owners, the attribution of the clinical event to the use of a particular drug ("suspect" drug), and the reporting of the ADE to the manufacturer of the suspected drug or directly to FDA. Data from these ADE reports are coded and entered into the computerized FDA/CVM ADE database. The reader should consult Chapter 58 for additional discussion of these topics.

It is important to remember certain caveats when using data from the FDA/CVM ADE database:

- For any given ADE report, there is no certainty that the suspected drug caused the adverse event. This is because veterinarians and animal owners are encouraged to report all suspected ADEs, not just those that are already known to be caused by the drug. The adverse event may have been related primarily to an underlying disease for which the drug was given, to other concomitant drugs, or may have occurred by chance at the same time the suspect drug was administered.
- Accumulated ADE reports should not be used to calculate incidence rates or estimates of drug risk. The Division of Surveillance is responsible for consolidation of all drug experience reports, necessary referrals and consultations, and preparation of the summary reports. The Divisions' ADE scoring system uses a modified Kramer scoring system (Bataller and Keller, 1999). In this system, each sign is separated from the other signs and scored according to previous experience with the drug, alternative etiological candidates (other causes), timing of the event, whether there was an overdose, whether the reaction continued or subsided with withdrawal of the drug, and whether a reaction recurred on reintroduction of a drug. Additional information on the criteria and responsibilities for the consolidation, screening, review, and evaluation of drug experience reports is further detailed in the CVM Program Policy and Procedures Manual section 1240.3522, Review and Evaluation of Drug Experience Reports (www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/PoliciesProceduresManual/ucm046832.pdf).)

Veterinarians and animal owners are encouraged to report adverse experiences and product failures to the government Agency that regulates the product in question. To access the FDA/CVM web site for information and forms that are needed to report adverse experience with veterinary drugs, the reader is referred to: www.fda.gov. Pretesting by the manufacturer and review of the data by the government does not guarantee absolute safety and effectiveness due to the inherent limitation imposed by testing the product on a limited population of animals. CVM encourages veterinarians and animal owners to contact the manufacturer of a suspect product. Withdrawal of an approved drug may be recommended if it is determined to be unsafe or ineffective.

The drug company marketing the drug product in question should be notified of the need to report an ADE for an FDA-approved animal drug. Drug company phone numbers can usually be obtained from product labeling. Technical services will ask a series of questions about the event, complete the FDA 1932 form, and forward the report to CVM. Alternatively, the report may be submitted directly to the FDA on Form 1932a.

Reports should preferably include a good medical history, all concomitant drugs the animal has been given, any recent surgical procedures, and as much in the way of clinical findings as is possible. Clinical findings may include veterinary exam, clinical chemistries, complete blood counts, urinalysis, fecal exams, radiographic results, and hemodynamic data such as blood pressure, any other pressure measurements in or around the heart, and neurological assessments.

"Veterinary Adverse Experience, Lack of Effectiveness or Product Defect Report" form FDA 1932a is a preaddressed, prepaid postage form that can be completed and dropped in the mail. This form may be obtained at: https://www.fda.gov/opacom/morechoices/fdaforms/FDA-1932a.pdf or by writing to:

ADE Reporting System
Center for Veterinary Medicine

US Food & Drug Administration
7500 Standish Place
Rockville, MD 20855-2773

The CVM can also be reached by phone at 1-888-FDA-VETS.

The Center may occasionally need more detailed information about an incident and the reporter may be called by a CVM staff veterinarian. The identities of all persons and animals is held in strict confidence by FDA and protected to the fullest extent of the law. The reporter's identity may be shared with the manufacturer or distributor unless requested otherwise. However, FDA will not disclose the reporter's identity to a request from the public, pursuant to the Freedom of Information Act. Information requested includes the reporter's name, address, phone number, and the brand name of the drug involved.

For information regarding CVM's surveillance program, the reader is referred to Chapter 58 of this textbook. Additional contact information includes:

- Animal biologics: vaccines, bacterins, and diagnostic kits**:** US Department of Agriculture 800-752-6255.
- Pesticides: topically applied external parasiticides: US Environmental Protection Agency 800-858-PEST

International Harmonization

Historically, the FDA has been concerned with the safety and effectiveness of animal drugs produced or marketed within the USA. The FFDCA requires that exported pharmaceuticals meet or exceed the standards for approval in the USA *or* for the country to which they are being exported. It also requires that animal drugs marketed in interstate commerce in the USA be approved through the same process by the FDA, regardless of where they are manufactured.

In addition to the US FDA, other countries have independently developed regulations and regulatory agencies that review safety, effectiveness, and purity of drugs that are intended for marketing within their specific jurisdiction for use in humans and/or animals. Although, internationally, the regulations share the common goal of protection of public health, the specific requirements and methodologies used in the evaluations of these products may differ.

When pursuing a broad international market, products may undergo multiple complete and autonomous reviews by regulating agencies in different countries or regions, and differences in the regulatory requirements between these agencies can hinder the development of new drugs and drug products. Regulatory differences can also increase product development expenses, complicate international trade (both of products and of foods derived from animals consuming these products), and can lead to duplication of animal studies. Growing globalization, emergence and spread of new animal diseases, and the international nature of the scientific community make it important to share innovation in animal treatment across borders, while maintaining standards for safety, quality, and effectiveness.

Internationally, formation of the European Community (the EC), and later the European Union (the EU) served as an impetus for the development of a shared regional process for the approval of new animal drugs. This set a pattern for harmonizing separate and diverse regulations among member countries. In 1990, the International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) was formed. This was followed by the International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products (VICH) in 1996. The VICH includes the USA, EU, and Japan as members, with the addition of Canada, New Zealand, Australia, and the Republic of South Africa as observers.

The objectives of the VICH are to:

- establish and monitor harmonized regulatory requirements for veterinary medicinal products in the VICH Regions, which meet high-quality, safety and efficacy standards, and minimize the use of test animals and costs of product development;
- provide a basis for wider international harmonization of registration requirements;
- monitor and maintain existing VICH guidelines, taking particular note of the ICH work program and, where necessary, update these VICH guidelines;
- ensure efficient processes for maintaining and monitoring consistent interpretation of data requirements following the implementation of VICH guidelines;
- by means of a constructive dialogue between regulatory authorities and industry, provide technical guidance enabling response to significant emerging global issues and science that impact on regulatory requirements within the VICH regions.

VICH Expert Working Groups, composed of scientific and technical experts representing each region, meet regularly to develop recommendations for more uniform standards for collection of preapproval and postapproval safety, purity, and effectiveness data. The goals of these Working Groups are to evaluate requirements of member agencies (within the context of current and emerging scientific knowledge), and to craft harmonized, science-based standards where ever possible. These standards are intended to maintain or enhance animal and public health and well being. Despite the challenges of reaching scientific and regulatory harmony, this process has led to a long list of approved and draft guidances, with more documents currently in development. These guidances are intended to allow the same studies to be used during the animal drug approval process of different regulatory agencies.

The FDA also participates in other international activities with a goal of protecting human and animal health in the global economy. During the early 1960s, the Food and Agriculture Organization (FAO) Conference and the World Health Organization (WHO) launched a joint effort to "protect the health of the consumers and ensure fair practices in the food trade". The primary product resulting from this program is the *Codex Alimentarius*, which literally means the "food code". This code represents a collection of food standards and guidance documents published jointly by the FAO and the WHO. Among these standards are maximum residual limits (MRLs) for veterinary drugs (MRLVD), as well as documents related to the use and control. The Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) is specifically responsible for addressing the MRLVDF (www.codexalimentarius.org/.)

Concluding Thoughts

Working through the provisions of the FFDCA, the FDA/CVM safeguards the health and productivity of food-producing animals and companion animal species. These activities represent an interaction of science, statistics, veterinary practice, and law. These efforts are complicated by an ever changing scientific, political, and veterinary medical landscape. Nevertheless, as changes occur, regulators work diligently in an effort to accommodate new issues, concerns, and technologies.

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Veterinary Pharmacy

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Introduction

A pharmacist's responsibility for providing patients with high-quality pharmaceutical care extends beyond the human species. Although colleges of pharmacy and licensing boards have focused almost exclusively on human pharmacotherapy, society expects an equally competent quality of pharmaceutical care and products to be provided for nonhuman family members. Veterinarians are trained to provide quality care and products for animal patients, but few pharmacists receive training in pharmacy school about veterinary medicine. Pharmacists may be asked more frequently to dispense medications for animals in their day-to-day practice and they must familiarize themselves with veterinary pharmacotherapy and develop a clinically and legally sound algorithm for processing veterinary prescriptions. Pharmacists who dispense medications for animals have an obligation to become familiar with important species differences with respect to pharmacotherapy and susceptibility to drug adverse reactions (Table 56.1). Because this process is very slowly evolving in the pharmacy profession, veterinarians should be well versed in pharmacy issues related to veterinary medical therapy. Although several of these issues have been introduced or discussed in other chapters of this book, this chapter describes a variety of pharmacy-related topics of which all veterinarians should be aware.

Regulatory Discretion for Extralabel Use

Advances in medical knowledge occur at a much faster rate than do drug product approvals. Since it is impossible for a drug company to test marketed drugs in all species at all doses and for all indications, it is difficult for veterinarians to adhere to the strict limitations of approved drug labels. Passage of the Animal Drug Amendment in 1968 significantly restricted use of drugs in animals to use only in those species for which the product was labeled with strict adherence to the labeled indication, dose, route of administration, and duration of therapy. Any use of a human-labeled drug in animals, for example, was considered illegal under the 1968 Amendment. Pursuant to enactment of this legislation, veterinarians were forced to break the law most of the time when using drugs in their patients. Physicians, on the other hand, were still allowed to use any drug at any dose for any indication in human patients.

Realizing the impracticality of strict interpretation of this law for veterinarians, the Food and Drug Administration published compliance policy guidelines (CPGs) in 1993 to show veterinarians the boundaries for regulatory discretion by FDA inspectors. Four main CPGs provided the boundaries for drug use by veterinarians: Extralabel Drug Use, Human Label Drug Use, Use of Drugs in Food Producing Animals, and Compounding for Animals (see www.fda.gov for summaries of these CPGs); however, the CPG for Compounding for Animals was rescinded in May 2015 and replaced with Guidance For Industry #230, Use of Bulk Drug Substances in Compounding For Animals. Chapter 55 of this book provides a background on the drug approval process by regulatory authorities and a summary of the legal control of veterinary drugs.

Need for Legal Extralabel use — AMDUCA

Although these CPGs provided veterinarians with a higher comfort level for using drugs in animal patients, extralabel use of drugs in animals was still illegal. The law did not change; CPGs only informed veterinarians of occasions when FDA inspectors would exercise regulatory discretion with respect to interpreting and enforcing extralabel drug use. In 1993, veterinarians, increasingly frustrated by being forced to break the law, demanded the same legal right physicians had in treating their patients. On October 22, 1994, the Animal Medicinal Drug Use Clarification Act (AMDUCA) was passed allowing veterinarians to legally use drugs extralabel under certain circumstances. AMDUCA went into effect December 9, 1996. Concurrently, Congress passed the

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Table 56.1 Information for pharmacists: excipients in medications or human drugs that may be harmful to pets

Drug/excipient/food	Species affected	Toxicity	Reference
Acetaminophen	Dogs, cats	Hepatotoxicity (dogs) and red blood cell oxidative injury (cats)	Campbell and Chapman, 2000, p. 31, 205
Alcohols	Dogs, cats birds	Central nervous system toxicity	Osweiler et al.,1976, p. 388
Avocado	Birds	Pulmonary congestion, nonsuppurative inflammation of the liver, kidney, pancreas, skin, and proventriculus	LaBonde, 2006
Benzocaine	Cats	Red blood cell oxidative injury, hemolytic anemia	Harvey, 2006
Chamomile	Cats	Emesis, diarrhea, depression, lethargy, epistaxis	
Chocolate	Dogs, birds	Cardiovascular and central nervous system stimulation	Campbell and Chapman, 2000, p. 106
Estrogen	Dogs	Bone marrow suppression	Campbell and Chapman, 2000, p. 245
Ethyl glycols (diethylene glycol, ethylene glycol)	Dogs, cats	Central nervous system toxicity, nephrotoxicity	Campbell and Chapman, 2000, p. 22, 127
Fat, fatty foods	Dogs	Increased risk of pancreatitis	
Garlic/onions	Dogs, cats	Hemolytic anemia	Warman, 2007
Grapes/raisins	Dogs	Renal toxicity	Warman, 2007
Macadamia nuts	Dogs	Neurotoxicity	Warman, 2007
Macrolide antibiotics, oral route	Horses, rabbits	Diarrhea, enteritis, colic	Papich, 2003
Methylene blue	Cats	Red blood cell oxidative injury, hemolytic anemia	Harvey, 2006
Nonsteroidal antiinflammatory agents for humans (naproxen, ibuprofen)	Dogs, cats	Gastrointestinal ulceration and perforation, nephrotoxicity	Campbell and Chapman, 2000, p. 148, 192
Pennyroyal	Cats	Hepatotoxicity	Wismer, 2007
Permethrin	Cats	Neuromuscular and central nervous system toxicity	Campbell and Chapman, 2000, p. 238
Phenazopyridine	Cats	Hepatotoxicity and red blood cell oxidative injury	Harvey, 2006
Phosphate enemas	Cats	Profound hypocalcemia	Wismer, 2017
Pseudoephedrine	Dogs, cats	Cardiovascular and central nervous system stimulation	Plumlee, 2004a
Raw yeast dough	Dogs	Alcohol poisoning, gastrointestinal dilatation and volvulus	Warman, 2007
Salt	Dogs, cats	Hypernatremia, central nervous system toxicity	Campbell and Chapman, 2000, p. 42
Tobacco products	Dogs, cats	Muscle weakness, twitching, depression, tachycardia, shallow respiration, collapse, coma, and cardiac arrest	Plumlee, 2004b
Xylitol	Dogs, birds	Profound hypoglycemia and hepatocellular necrosis	Wismer, 2006

Animal Drug Availability Act, significantly expediting the process by which FDA approved products intended for use in animals. Passage of both laws was intended to significantly improve the ability of veterinarians to best treat nonfood-producing animal patients.

Animal Medicinal Drug Use Clarification Act

On October 22, 1996, the Animal Medicinal Drug Use Clarification Act of 1994 was enacted into law, allowing veterinarians to prescribe extralabel use of veterinary and human drugs for animals under specific circumstances and also codified compounding for animal patients provided the starting ingredients were FDA-approved drug products. The key extralabel provisions of the Animal Medicinal Drug Use Clarification Act are provided in Table 56.2. (A copy of the AMDUCA Extra Label Drug Use Guidelines Brochure is available in the February 15, 1998, issue of the Journal of the American Veterinary Medical Association (JAVMA) or may be obtained from the AVMA website: www.avma.org.) Some drugs are strictly prohibited from administration to food animals because a safe level of residue in food products cannot be identified (Table 56.3). Additional provisions

Table 56.2 Requirements for extralabel drug use (ELDU) in animals

- ELDU is permitted only by or under the supervision of a veterinarian.
- ELDU is allowed only for FDA-approved animal and human drugs.
- A valid veterinarian/client/patient relationship is a prerequisite for all ELDU.
- ELDU is for therapeutic purposes only (animal's health is suffering or threatened), not for production use.
- Rules apply to dosage for drugs and drugs administered in water. ELDU in feed is prohibited.
- ELDU is not permitted if it results in a violative food residue, or any residue that may present a risk to public health. FDA prohibition of a specific ELDU precludes such use (Table 56.3).

of the AMDUCA legislation require proper dispensing and labeling of prescribed drugs for animals (Tables 56.4 and 56.5).

Classification of Drugs

Drug

A chemical can be considered a drug if it meets one of the following criteria: (Chapter 5 of this book covers special considerations on pharmaceutics.)

1) It is an article recognized in one of the official compendia, i.e., the *United States Pharmacopoeia/National Formulary* or the official *Homeopathic Pharmacopoeia* of the United States or their supplements.
2) It is an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease in man or other animals.
3) It is an article other than food intended to affect the structure or any function of the body of man or other animals.
4) It is an article that is intended to be used as a component of an item falling into one of the three categories above.

Table 56.3 Drugs prohibited for extralabel use in food animals

- Chloramphenicol
- Clenbuterol
- Diethylstilbestrol (DES)
- Dimetridazole
- Ipronidazole
- Other Nitroimidazoles
- Furazolidone (except for approved topical use)
- Nitrofurazone (except for approved topical use)
- Sulfonamide drugs in lactating dairy cows (except approved use of sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine)
- Fluoroquinolones
- Glycopeptides (example: vancomycin)
- Phenylbutazone for female dairy cattle over 20 months of age

Table 56.4 Record requirements for drugs dispensed for animals

- Identify the animals, either as individuals or a group
- Animal species treated
- Numbers of animals treated
- Condition being treated
- The established name of the drug and active ingredient
- Dosage prescribed or used
- Duration of treatment
- Specified withdrawal, withholding, or discard time(s), if applicable, for meat, milk, eggs, or animal-derived food
- Keep records for 2 years
- FDA may have access to these records to estimate risk to public health

This definition does not differentiate between prescription drugs and nonprescription drugs, nor does it distinguish legal or lawful drugs from illicit ones. Because of this broad definition, *any substance that is used to treat an animal can be ultimately considered to be a drug and can be regulated and actionable as such.*

Legend ("Prescription") Drugs

Prescription drugs are limited to dispensing by or upon the order of a licensed prescriber ("prescription") because they are habit forming, are toxic, or have potential for harm. These drug labels contain the following warnings identifying them as legend drugs:

- *Veterinary Legend*: "Caution: Federal law restricts this drug to use by or on the order of a licensed veterinarian."
- *Human Legend:* "Rx only."

Legend (prescription) drugs *cannot* be dispensed without a prescription and may only be prescribed and dispensed within the confines of a valid veterinarian–client–patient relationship (VCPR) (Table 56.6). Because of the requirement of a valid VCPR, if a veterinarian has not examined the animal, he/she cannot prescribe legend

Table 56.5 Label requirements for drugs prescribed for animals

Name and address of the prescribing veterinarian
Established name of the drug
Any specified directions for use including the class/species or identification of the animal or herd, flock, pen, lot, or other group; the dosage frequency and route of administration; and the duration of therapy
Any cautionary statements
Your specified withdrawal, withholding, or discard time for meat, milk, eggs, or any other food

Table 56.6 A valid veterinarian–client–patient relationship (VCPR)

- The veterinarian has assumed the responsibility of making medical judgments regarding the health of the animal(s) and the need for medical treatment, and the client (owner or other caretaker) has agreed to follow the instructions of the veterinarian.
- There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means that the veterinarian has recently seen and is personally acquainted with the keeping and care of the animal(s) by virtue of an examination of the animal(s), and/or by medically appropriate and timely visits to the premises where the animal(s) are kept.
- The practicing veterinarian is readily available for follow-up in case of adverse reactions or failure of the regimen of therapy.

drugs for use in that animal. Likewise, a veterinarian cannot "fill a prescription" for an animal unless he/she has a valid VCPR with that animal. Veterinarians filling prescriptions for other veterinarians are considered to be practicing "pharmacy" and are subsequently subject to action by state pharmacy regulatory boards.

Over-The-Counter ("Nonprescription") Drugs

Over-the-counter (OTC) drugs are also considered "nonprescription" drugs. These are familiar to veterinarians and pet owners because they are available for human use in retail outlets such as pharmacies, markets, and grocery stores. Such OTC products can also be found for animals in the same retail outlets as for humans as well as in pet and feed stores. These drugs have been recognized by experts as safe and effective and bear extensive labeling that renders them safe for use by laypersons and are sold "over the counter," without a prescription.

All OTC products must be used *precisely* as labeled just as legend drugs. Use outside of these specifications constitutes extralabel use and the aforementioned guidelines should govern this use. Pharmacists must not make recommendations for use of human OTC drugs in animals unless so directed by a veterinarian. Veterinarians should avoid repackaging OTC medications for dispensing because reproducing the required labeling that is comprehensive enough for safe use by a layperson is difficult and dangerous.

VFD Drugs

A separate and unique category of drugs are those that fall under the Veterinary Feed Directive (VFD). A "VFD drug" is a drug intended for use in or on animal feed, which is limited to use under the professional supervision of a licensed veterinarian. A VFD drug is not a prescription drug, but is a written (nonverbal) statement issued by a licensed veterinarian in the course of the veterinarian's professional practice that authorizes the use of a VFD drug or combination VFD drug in or on an animal feed. Because there are new regulations that pertain to the VFD taking effect on January 1, 2017, this topic is discussed in more detail in Chapter 55 and 59 in the regulatory section of this book.

Controlled Substances

Controlled substances ("narcotics") are defined and monitored by the Drug Enforcement Authority (under jurisdiction of the Controlled Substances Act of 1970) and are divided into five schedules according to potential for abuse. An example of the schedules used for opiate drugs is provided in Chapter 13. Other controlled drugs are anesthetics and sedatives listed in Chapters 12 and 14. These drugs are strictly controlled by federal and state law and specific requirements for administering, dispensing, and prescribing are addressed in Section Prescribing Controlled Substances. Individual states are allowed to have more strict requirements than the federal (DEA) scheduling.

Compounded Drugs

Compounded drugs are mixtures of approved dosage forms or drugs formulated from bulk chemicals that are not approved by FDA for use as drugs in the United States. Veterinarians may compound items for their own use or write prescriptions for their patients for some of these active pharmaceutical ingredients to be used in preparing compounds by licensed pharmacists. These active pharmaceutical ingredients (e.g., potassium bromide, cisapride, diethylstilbestrol) are considered as drugs when used for therapeutic purposes and are recognized by FDA to be essential in the treatment of some companion, nonfood animals. As many of these drugs have been withdrawn from the market because of human safety hazards, the FDA has published a "negative" list (Drugs Withdrawn for Safety or Efficacy Reasons) for human compounding describing the drugs that are either prohibited for use in humans or are restricted to small dosages. Note that some of the drugs (e.g., cisapride and diethylstilbestrol) may still be compounded for nonfood animals such as companion animal pets but never for food animals or humans.

Current Status of Compounded Veterinary Drugs

Drug compounding has always been an important component of veterinary medicine. Historically, veterinarians have prepared concoctions, mixtures, and remedies for their patients because there were few approved formulations on the market for animals. Now, there are more available drugs for animals, and pharmaceutical science has provided for a better understanding of the factors contributing to poor drug bioavailability, instability, and physical incompatibility. Over the last several years, questions concerning the practice of compounding have been raised, particularly with respect to stability, purity, and strength when the original dosage form of the drug is altered. *(Note that the term "potency" used in some publications has been replaced by "strength" in this chapter because it is a more accurate term. Potency is used to describe the biological activity of a medication in a patient, and is not a measure of drug concentration.)*

Compounding is the alteration of the original drug dosage form for the purposes of ease of administration or because the original dosage form is unsuitable for the purpose intended. According to the United States Pharmacopeia (USP), compounding involves the preparation, mixing, assembling, packaging, and labeling of a drug or device in accordance with a licensed practitioner's prescription. The USP chapter on pharmacy compounding (Chapter <795>: Pharmaceutical Compounding—Nonsterile Preparations) states that "compounding is an integral part of pharmacy practice and is essential to the provision of health care" (USP-NF, 2015a).

Compounding does not include the preparation of a drug by reconstitution or mixing that is according to the manufacturer's instructions on an approved human or veterinary drug product.

The FDA issued a Compliance Policy Guide (CPG) for compounding drugs for use in animals in 2003 (FDA-CVM, 2003b). The 2003 version of the CPG provided guidance to FDA's staff with regard to the compounding of animal drugs by veterinarians and pharmacists for use in animals. However, in 2015 the agency withdrew Compliance Policy Guide Section 608.400 "Compounding of Drugs for Use in Animals" because it is no longer consistent with the FDA's current thinking on these issues. The FDA has prepared new guidance, referred to as "Guidance for Industry, or GFI #230". (Available at: http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm446846.htm). At the time of this writing, the draft has not yet been finalized; therefore, it is not possible to indicate in this chapter how compounding will be regulated in the future. However, from the draft guidance, it is clear that the FDA intends to demonstrate regulatory discretion for use of bulk drug substances for compounding and intends to place stronger limits on the compounding from bulk sources. *Bulk drug substances* are defined as active ingredients used in the manufacture of finished dosage forms of the drug. Bulk drug substances are also referred to as *active pharmaceutical ingredients* (APIs). Current law does not permit compounding of animal drugs from bulk drug substances, but the FDA recognizes that there are limited circumstances when an animal drug compounded from bulk drug substances may be an appropriate treatment option. FDA's GFI #230 outlines specific conditions under which the agency generally does not intend to take action against state-licensed pharmacies, veterinarians, and facilities registered as outsourcing facilities when drugs are compounded for animals from bulk drug substances. It is anticipated that the new FDA Guidance on compounding from bulk drug substances will allow outsourcing facilities to prepare compounds from a limited list of bulk drug substances. Compounding from such a list by outsourcing facilities may be allowed in the absence of a prescription for an individual animal in instances such as drug shortages, or for when emergent use precludes waiting for a compound to be prepared pursuant to an individual prescription for an individual animal. *Outsourcing Facilities* are defined in The Drug Quality and Security Act, signed into law on November 27, 2013. This law created a new section 503B in the Food Drug and Cosmetic Act (FDCA). Under section 503B, a compounder can become an "outsourcing facility." The law defines an "outsourcing facility" as a facility at one geographic location or address that is engaged in the compounding of sterile drugs; has elected to register as an outsourcing facility; and complies with all of the requirements of section 503B. The FDA recognizes the importance of compounding in veterinary practice, but also must ensure that compounded drugs do not cause harm to the treated animals or their caregivers, that compounded preparations are bioavailable, effective, stable, and potent, and that compounded preparations do not cause drug residues in food animals. The proposed guidance mentioned above specifically refers to compounding from unapproved bulk drug substances.

FDA regulations permit the compounding of formulations from approved animal or human drugs under the current federal code: 21 CFR 530.13 (AMDUCA). The FDA is concerned that some compounding by veterinarians and pharmacists from bulk drug substances is performed to circumvent the usual drug approval process. Some activities performed under the guise of compounding (e.g., mass preparation of compounds that are wholesaled to veterinarians with no patient identified at the time of compounding, *dispensing* of compounds intended only for *administration* by the veterinarian in the office, or compounding less expensive copies of commercially available approved drugs) mimics exactly the manufacturing and distribution of what FDA defines as "new animal drugs." A court ruling by the 5th Circuit of Appeals (*Medical Center Pharmacy vs. Mukasey*, July 2008) reasserted that compounds prepared for animals outside of the provisions of AMUDCA fit the description of new animal drugs. New animal drugs must undergo the long and arduous FDA approval process to ensure the safety of the public health. Compounded products have not been subject to this scrutiny to determine the safety, efficacy, strength, purity, and stability required of drug manufacturers in order to receive FDA approval for marketing. Pharmacies are held to relevant USP Compounding standards by their state boards of pharmacy; however, under no circumstances can compounded products be manufactured or wholesaled. Licensed veterinary distributors who wholesale compounds from compounding pharmacies are in direct violation with licensure as granted by state licensing agencies (usually the Department of Revenue). Compounds must be prepared by pharmacists pursuant to a valid prescription order for an individual patient or prepared by outsourcing facilities for use in a veterinary practice (“office use”).

Potential Problems from Compounded Drugs

Because many drugs are not in a form that is ideal for the species being treated, either due to body size, taste preferences, or species-specific metabolic intolerances, commercially available drug products have been altered to make a more convenient and palatable oral dose form. However, when protective coatings on tablets or capsules are disrupted, and suspending or solubilizing vehicles are diluted or changed, the bioavailability and stability of the product may be compromised. (See Chapter 5 of this book for more detailed information on pharmaceutics.) In some instances, the only change is a slight alteration of pH. However, according to the USP (2015c), “improper pH ranks with exposure to elevated temperature as a factor most likely to cause a clinically significant loss of drug. A drug solution or suspension may be stable for days, weeks, or even years in its original formulation, but when mixed with another liquid that changes the pH, it degrades in minutes or days. It is possible that a pH change of only one unit could decrease drug stability by a factor of ten or greater.” Addition of a water-based solution to a product to make a liquid solution or suspension can hydrolyze some drugs (beta-lactams, esters). Some drugs undergo epimerization (steric rearrangement) when exposed to a pH range higher than what is optimum for the drug (for example, this occurs with tetracycline at a pH higher than 3). Other drugs are oxidized, catalyzed by high pH, which renders the drug inactive. Drugs most likely to be subject to oxidation are those with a hydroxyl group bonded to an aromatic ring structure. Oxidation may occur from exposure to light and oxygen during reformulation and mixing. Oxidation is catalyzed by high pH and usually leads to drug inactivation. Other factors contributing to instability and decrease in bioavailability may be through the addition of sugars and starches to an oral suspension. For example, it is well documented that the presence of sugar significantly decreases the stability of oral suspensions of atenolol and pyrimethamine, and the addition of methylcellulose to solutions of pyridostigmine will significantly decrease the oral bioavailability of pyridostigmine resulting in potential harm to the patient receiving these medications.

Table 56.7 Signs of drug instability of compounded formulations

Liquid dose forms
Color change (pink or amber)
Signs of microbial growth
Cloudiness, haze, flocculent, or film formation
Separation of phases (e.g., oil and water, emulsion)
Precipitation, clumping, crystal formation
Droplets or fog forming on inside of container
Gas or odor release
Swelling of container

Solid dose forms
Odor (sulfur or vinegar odor)
Excessive powder or crumbling
Cracks or chips in tablets
Swelling of tablets or capsules
Sticking together of capsules or tablets
Tackiness of the covering of tablets or capsules

Veterinarians and pharmacists are obligated to be cognizant of the potential for interactions and interference with stability (Table 56.7). Oxidation is often visible through a color change (color change to pink or amber, for example). Loss of solubility may be observed through precipitation. Some drugs are prone to hydrolysis from moisture. A rule-of-thumb for veterinarians is that if a drug is packaged in blister packs or moisture-proof barrier, it is probably subject to loss of stability and strength if mixed with aqueous vehicles. If compounded formulations of solid dose forms show cracking or “caking,” or swelling, the formulation has probably accumulated moisture and may have lost strength over time. Another rule-of-thumb is that if the original packaging of a drug is in a light-resistant or amber container it is probably prone to inactivation by light. Vitamins, cardiovascular drugs, and phenothiazines are labile to oxidation from light during compounding. Also, as a general rule, if an antibiotic is available in a powder that must be reconstituted in a vial or oral dispensing bottle prior to administration, it is probably unstable for long periods of time and should also not be mixed with other drugs.

Examples of Potential Problems

There are published studies in which drugs for veterinary patients have been tested for strength over time or stability under the conditions used during compounding. In a commercial formulation, the inactive ingredients and excipients are added to drug formulations to ensure the stability of the drug; provide an optimum chemical environment, pH; or increase the ease of packaging or handling. However, adding other chemicals, flavorings, or vehicles, or interfering with protective coatings of tablets may affect the stability of the drug, decreasing strength, oral absorption, and efficacy. There are published formulas in compounding journals, and texts, but few of these formulations have been tested for safety, efficacy, bioavailability, stability, strength, and purity for use in the target species. Veterinarians are responsible for the risk to an animal, or person handling the medication, when they prescribe compounded preparations. Pharmacists and veterinarians preparing compounds for animal patients are obligated to be in compliance with USP compounding standards. They have an obligation to request evidence from compounding pharmacists about the stability and strength of formulations prepared for their patients. When veterinarians compound formulations in their own practices, they should be cognizant of the potential interactions and alterations that may compromise the stability and strength of the active ingredient and should not attempt compounding without proper training, equipment, and quality assurance techniques

There are published examples in which drug stability and efficacy have been compromised through compounding. For example, when omeprazole was compounded for oral use in horses, it was not as effective for treating gastric ulcers as the commercial formulation registered for horses (GastroGard) (Nieto et al., 2002). Systemic bioavailability and strength of the compounded formulation was not as high as for the proprietary product. Omeprazole is known for its instability unless administered in the original formulation intended for horses or people. On the other hand, when the FDA-approved equine paste (GastroGard ®) was compounded in oil for dogs and cats, it retained the original strength for 6 months (unpublished data from laboratory of MG Papich).

Fluoroquinolone antibiotics are frequently modified for administration to exotic animals and horses. The compatibility of enrofloxacin and orbifloxacin with flavorings, vehicles, and other ingredients has been evaluated. With few exceptions, this class of drugs is compatible with most mixtures and remarkably stable. Enrofloxacin retained strength when the FDA-approved product was mixed with various vehicles and flavorings for exotic animals (Petritz et al., 2013). A notable exception is the chelation of enrofloxacin with iron and aluminum-containing products (e.g., antacids, sucralfate, mineral supplements, iron-containing molasses), in which a significant portion of the medication may become unavailable for absorption. It has also been observed that certain mixtures and flavorings may be incompatible with fluoroquinolones if they contain metal ions that are known to cause chelation. For example, when crushed orbifloxacin tablets were mixed with a vitamin and mineral supplement (Lixotinic) that is sometimes used as a flavored vehicle for oral administration of drugs, the strength of orbifloxacin was decreased from its original concentration by 50%. (Lixotinic contains 2.5 mg/ml iron.) Other flavorings and vehicles (for example, corn syrup, regular molasses, fish sauce, and Syrpalta) had no effect.

Antifungal drugs are subject to instability if not maintained at an optimum pH and formulation conditions. Itraconazole is frequently compounded from the bulk chemical or the proprietary capsules. However, itraconazole must be complexed onto cyclodextran in order to be orally bioavailable, and as bulk chemical preparations of itraconazole are not complexed in this fashion, they have not been found to be orally bioavailable in animal patients (Mawby et al., 2014). Itraconazole may also adsorb to plastic and glassware during compounding, decreasing the predicted strength of the finished preparation.

When doxycycline hyclate was compounded from FDA-approved tablets in an aqueous vehicle, it retained strength for only 7 days. After 7 days it degraded and showed a change in color and consistency (Papich et al., 2013).

Aminoglycoside antibiotics (gentamicin, tobramycin, kanamycin) are inactivated when admixed with other antibiotics, particularly beta-lactams. This interaction is greatest from carbenicillin, followed by ticarcillin, penicillin G, and ampicillin. Loss of strength by as much as 50% can occur within 4 to 6 hours. This interaction is a potential problem when antibiotic mixtures are prepared in a vial or fluid administration set and dispensed to be used several hours later. This interaction does not occur intravascularly at therapeutic concentrations in the patient because the drugs are diluted out in plasma and body fluids (Bowman et al., 1986), but visual precipitation of these agents in IV administration sets is commonly reported when they are mixed.

Drugs formulated as acids – such as the hydrochloride form of basic drugs – are formulated to maintain their solubility in aqueous solutions. However, when these formulations are mixed with other drugs that are more basic, or added to basic vehicles, drug precipitation may occur.

Several drugs are not soluble in aqueous vehicles. Therefore they are dissolved in organic solvents (propylene or ethylene glycol, for example) or alcohols. These are notoriously unpalatable to some animals, particularly cats. However, if these formulations are diluted in aqueous fluids, precipitation may occur. When these are stored at home by the pet owner, precipitation of the drug to the bottom of the container results in dilute oral dosing when the container is sampled from the top, and highly concentrated formulation when the container is sampled from the bottom (assuming that the precipitate at the bottom can be resuspended). This also may be observed when mixing some drugs in aqueous fluids. For example, if diazepam solution (which contains propylene glycol and alcohols) is diluted in saline solution or Lactated Ringer's solution, precipitation occurs.

Compounded Transdermal Medications for Pets

Because of convenience, ease of administration, and therapeutic success with some transdermal drugs (antiparasitic agents and fentanyl), there is considerable interest in formulating a wide range of other drugs for use by this route. Historically, transdermally administered drugs were formulated for single-dose delivery in a liquid preparation, or for continuous delivery out of a drug-releasing matrix or "patch." A compounding pharmacist (Marty Jones, PharmD) discovered a penetration-enhancing drug vehicle in the 1990s, and application of this delivery system to drugs used in animals rapidly caught on. A quick Internet search of "compounding pharmacy + transdermal" will reveal over 11,000 advertisements from compounding pharmacies who provide drugs in transdermal delivery gels. Noninvasive, nonstressful, palatable drug delivery is an attractive option for many veterinary patients, especially cats. Consequently, many veterinarians are eager to try this dosage form on fractious or fragile patients who should not be (or do not want to be) stressed during medication. While transdermal drug delivery is appealing, there is only one drug (methimazole) that was shown to produce therapeutic benefits when compounded and administered via the transdermal route in cats. The skin is a formidable barrier to drug penetration, and drug absorption into systemic circulation is difficult (Riviere and Papich, 2001)(see Chapter 47 of this book). In order to facilitate drug transport across skin, drugs need to be small in molecular weight and placed in a biphasic vehicle that can propel the drug across the various lipophilic and hydrophilic layers of the skin. Passage of the drug across the skin relies on the thickness of the skin, the partition coefficient of the drug, the diffusion coefficient of the skin, and the concentration of drug in solution. The partition coefficient, the diffusion coefficient, and the thickness of the skin are not alterable; therefore, transdermally applied drugs must be placed in high concentrations in vehicles that accommodate the various coefficients of diffusion. There are few drugs that can meet these criteria to be successfully absorbed by this route.

One commonly used penetration enhancer is pleuronic lecithin organogel (PLO), which is lecithin (derived from eggs or soybeans) mixed with isopropyl palmitate and a poloxamer (Pluronic). The ingredients in PLO act as surfactants, emulsifiers, and solubilizing agents to escort drug across the skin into systemic vasculature. There are many other penetration-enhancing vehicles (e.g., Lipoderm, Van Penn) utilized to compound transdermal preparations, but at the time of writing, there are no FDA-approved formulations that utilize these penetration-enhancing vehicles to deliver systemic drugs. Most FDA-approved transdermal drugs for people are available as patch delivery systems (e.g., patches containing fentanyl, lidocaine, or buprenorphine), or are single-dose applications of parasiticides for animals. Compounded transdermal preparations are formulated in high concentrations so that a therapeutic dose may be delivered in 0.1–0.2 ml, which is applied to a hairless area and rubbed in until no residue remains on the skin surface. Because the drug must be in solution in order to penetrate skin, many drugs are logically precluded from transdermal administration because they are not soluble in concentrations high enough to deliver a dose in 0.1–0.2 ml (e.g., any drug dosed at 10 mg/kg requires a transdermal concentration of at least 500 mg/ml to deliver a 0.1-ml dose to a 5-kg cat). The majority of drugs are not soluble at these high concentrations.

Single-dose pharmacokinetic studies for transdermally administered drugs have demonstrated that absorption was incomplete, nonexistent, or highly inconsistent among study cats, and that after single doses, bioavailability was low compared to a single oral dose. There have been an even smaller number of chronic dosing safety and efficacy studies for transdermally administered drugs, and few of those demonstrated positive evidence of efficacy. Transdermally administered drugs examined so far have included methimazole, amlodipine, glipizide, dexamethasone, buspirone, amitriptyline, metoclopramide, atenolol, fentanyl, morphine, fluoxetine, ondansetron, theophylline, and diltiazem. Methimazole was not absorbed well according to a pharmacokinetic study (Hoffman et al., 2002; Trepanier, 2002), but produced clinical efficacy with repeated transdermal application in other studies (Hoffman et al., 2001). It is suspected that efficacy from repeated doses of transdermal methimazole is attributed to the cat rubbing their paw on the ear, then licking the medication from the paw producing oral absorption. Transdermal amlodipine produced a change in blood pressure in a small study in hypertensive cats (Helms, 2007), but it is known that blood pressure response from amlodipine in cats can be highly variable. Absorption of atenolol from a transdermal preparation in cats was low and inconsistent and did not produce therapeutic blood levels of atenolol after a week of chronic dosing at an equivalent oral dose (MacGregor et al., 2008). The remaining drugs were examined as single-dose pharmacokinetic studies and showed poor bioavailability as compared to oral dosing and a high degree of intrasubject variation in drug absorption.

A major concern for use of transdermally administered drugs, for which there is no safety, efficacy, or strength evidence, is the risk of poor absorption or decreased stability of the formulated drug. For example, unstable or poorly absorbed transdermal antibiotics may result in therapeutic failure or increase the risk of antimicrobial drug resistance. Misinterpretation of single-dose pharmacokinetic studies might lead prescribers to increase transdermally administered drug doses, leading to accumulation of drug and severe toxicity. For example, the transdermal bioavailability of amitriptyline was 10% compared to oral dosing in a single dose pharmacokinetic study. Several veterinarians erroneously interpreted these results to mean that transdermal amitriptyline should effectively be administered at 10 times the oral dose. Other poor candidates for transdermal administration include drugs that are known to be contact irritants or phototoxins. In addition to bioavailability rationale, veterinarians evaluating drugs for transdermal application should also consider the local damage caused by these drugs (e.g. clopidogrel, doxycycline, fluoxetine, enrofloxacin) when applied to thin, hairless skin (e.g. pinnae). Obviously, there also is considerable risk for absorption by the human caregiver. Drugs toxic to humans (e.g., chloramphenicol, diethylstilbestrol, carprofen, digoxin anticancer agents) are extremely poor candidates for transdermal drug administration. Similarly, caregiver health and lifestyle must also be considered when prescribing transdermal drugs for pets. For example, a hypothyroid owner is a poor candidate to administer transdermal methimazole to her hyperthyroid cat, and a long-distance truck driver subject to periodic drug testing is a poor candidate for administration of transdermal opiates to his pet in pain.

Compounding Guidelines for Veterinarians and Veterinary Pharmacists

The future FDA-CVM guidance on compounding (GFI #230) will define the extent of compounding from bulk substances that will be allowed after this guidance if finalized. In the meantime, there are restrictions that apply to compounding in general, and each state may have more restrictive requirements than federal law. In the meantime, compounded therapies should be prepared from a commercially available formulation, if a suitable approved product exists, and compounding pharmacies should follow existing standards and guidelines.

The United States Pharmacopeia (USP), a national standard-setting organization for pharmaceuticals, lists specific standards for pharmaceutical compounding in Chapters <795> Pharmaceutical Compounding—Non-Sterile Preparations, and <797> Pharmaceutical Compounding—Sterile Preparations, (USP-NF, 2015a). One important standard for compounded preparations is that the final strength of the finished preparation is not less than 90.0% and not more than 110% of the theoretically calculated and labeled quantity of active ingredient per unit weight or volume. There are also guidelines available for stability considerations in the chapter on Observing Products for Evidence of Instability (Chapter <1191>) (USP-NF, 2015b). Generally, the beyond-use dating (BUD) for a nonaqueous solid compounded dosage form should not be later than the time remaining until the expiration date of the shortest-dated ingredient or 6 months, which ever is shorter. For water-containing oral formulations the beyond-use date is not later than 14 days stored at controlled cold temperatures, and for nonaqueous liquid formulations USP Chapter <795> allows for the beyond-use date to be no later than 180 days. For water-containing topical formulations, the beyond use date is 30 days at controlled room temperature. These limits may be exceeded when there is supporting valid scientific data that applies to the specific compounded formulation.

The Society of Veterinary Hospital Pharmacists, an organization of academic veterinary teaching hospital pharmacists, has published a position statement on Compounding For Animal Patients that may be consulted by pharmacists or veterinarians for further guidance (http://svhp.org).

Finally, veterinarians should seek the services of a Pharmacy Compounding Accreditation Board (PCAB) accredited pharmacy when prescribing compounds. Formed in 2004 by eight national pharmacy organizations, the nonprofit PCAB runs a voluntary accreditation program to ensure quality standards for compounding pharmacies. Once a pharmacy applies for PCAB accreditation, they must undergo a rigorous standards evaluation that is then validated by multiple site visits and inspections by PCAB surveyors. If a pharmacy meets the incredibly rigorous accreditation standards, a veterinarian can be assured that the pharmacy is legally and ethically impeccable and that its compounds are of the highest possible quality. At the time of this writing, PCAB has accredited more than 300 pharmacies in the United States. PCAB maintains an interactive map of accredited pharmacies in each state, list of accredited compounding pharmacies on their website, or veterinarians can contact PCAB's executive director to find an accredited pharmacy in their state. Further information regarding PCAB can be reached at: http://www.achc.org/compounding-pharmacy.html.

Unapproved Drugs Available by Importation

There was a mechanism in the past whereby a veterinarian could obtain an unapproved drug or dosage form through importation from other countries by filing a Medically Necessary Personal Veterinary Import form with FDA-CVM. This mechanism is no longer sanctioned by the FDA-CVM.

Prescribing Controlled Substances

A veterinarian who administers, dispenses, or prescribes controlled substances in the course of his practice *MUST* register with the Drug Enforcement Authority (DEA). This requires submission of an application, which may be obtained from the DEA. Practitioners must have a separate registration for every practice site employed. State regulations also must be followed and practitioners must contact their state authorities to determine the requirements. Some states have stricter regulations that the Federal drug schedules. Veterinarians can find out more about the DEA drug schedules and handling of controlled substances in their practice by viewing the DEA web site (see: http://www.deadiversion.usdoj.gov/schedules/).

Prescribing controlled substances requires submission of appropriate forms and maintaining records for controlled substances. Documentation must be made in the patient's medical record of all controlled substances administered, and such records must be stored in a readily retrievable fashion for 3 years. Appropriate entries must be made for waste or disposal of unused portions of controlled substance and witness is required for disposal. Records must be kept of all controlled substances dispensed, and such records must be stored in a readily retrievable fashion for 3 years. While the law does not specifically require a dispensing record separate from the medical record, a separate recordkeeping system provides more readily retrievable dispensing records than does the patient's medical record. A separate dispensing log does *NOT* replace the need to document dispensing of drugs in the medical record because these drugs are still restricted to the order of a licensed practitioner. It is also recommended that Schedule II dispensing records be maintained separately from other records.

Prescribing Records

Practitioners are not required to keep records of prescriptions written for controlled substances, but it is in the best interest of the veterinarian to make a note of such prescriptions in the patient's medical record to provide for a complete patient medical profile.

Inventory Records

All DEA 222 order forms for Schedule II orders as well as other commercial invoices accompanying controlled drugs must be signed and dated, and items must be counted, noted, and stored in a readily retrievable file for 3 years. Schedule II records must be stored separately from Schedule III-V records.

A complete and accurate inventory of all stocks of all controlled substances must be taken every 2 years on the anniversary of the practitioner's initial DEA registration. The written inventory should contain the following information:

- the name, address, and DEA registration number of the registrant;
- the date and time the inventory is taken;
- the signature of the person taking inventory;
- an indication that the inventory is maintained for at least 2 years at the location appearing on the registration;
- an indication that inventory and other records of Schedule II drugs are maintained separately from other drugs.

Storage and Security of Controlled Substances

Controlled substances "must be stored in a securely locked, substantially constructed cabinet." If there is loss or theft, upon discovery of loss of theft of controlled substances, the registrant must immediately notify the region office of the DEA and then must complete DEA form 106 describing the loss. If a theft is verified, the local police department must also be notified.

Dispensing Medications Intended to Go Home with a Patient

Many state and federal legal requirements exist for the act of dispensing a drug to go home with a patient. Any prescription medications that leave a practice to go home with a patient must be labeled with the information indicated in Table 56.8.

Any medications that leave a practice to go home with a patient should be packaged in a child-resistant container as described by the Poison Prevention Packaging Act.

Table 56.8 Drug dispensing labeling requirements

- Name, address, and phone number of the dispensing facility
- Name of client
- Animal identification (name and species)
- Date dispensed
- Full directions for use
- Name, strength, and quantity of drug dispensed
- Name of prescribing veterinarian
- For controlled substances, the label must also contain the message "Caution: Federal law prohibits transfer of this drug to any person other than the patient for whom it was prescribed."

Table 56.9 Requirements for writing a prescription for noncontrolled substances

The elements listed below are required by law to be included on the written prescription document:

- Printed or stamped name, address, and telephone number of the licensed practitioner
- Legal signature of the licensed practitioner
- Name and strength of drug
- Directions for use
- Full name and address of the client
- Animal identification (name and/or species)
- Cautionary statements including, if applicable, withdrawal times for food animals
- Number of refills, if any

Table 56.10 Requirements for writing a prescription for controlled substances

The elements listed below are required by law to be included on the written prescription document:

- Printed or stamped name, address, and telephone number of the licensed practitioner
- DEA registration number of the licensed practitioner
- Legal signature of the licensed practitioner followed by printed name of the practitioner
- Name and strength of drug
- Directions for use
- Full name and address of client
- Animal identification (name and/or species)
- Cautionary statements including, if applicable, withdrawal times for food animals

No refills are allowed on Schedule II prescriptions and refills are limited to 5 times or 6 months (whichever comes first) on prescriptions for drugs in Schedules III–V.

The Act applies only to drug dispensed for human use, but should be followed to prevent unintentional human poisonings from animal drugs. Medications dispensed in nonchild-resistant containers should be adequately labeled with auxiliary labels indicating "Caution: Package not child resistant" and "Keep out of reach of children." Other auxiliary labels detailing important information such as "shake well," "refrigerate," "not for injection," etc. should also be affixed to the container.

Table 56.11 Officially recognized Latin abbreviations used in prescription writing

a.c.	before meals	ml	milliliter
a.d.	right ear	o.d.	right eye
a.s.	left ear	o.s.	left eye
a.u.	both ears	o.u.	both eyes
amp	ampoule	p.c.	after meals
b.i.d.	twice daily	p.o.	by mouth
cc	cubic centimeter	p.r.n.	as needed
c	with	q.	every
cap	capsule	q4h	every 4 hours, etc.
disp	dispense	q.i.d.	four times daily
g or gm	gram	q.s.	a sufficient quantity
gtt(s)	drop(s)	Sig.	directions to patient
h	hour	SQ	subcutaneously
h.s.	at bedtime	stat	immediately
IM	intramuscularly	susp	suspension
IP	intraperitoneally	tab	tablet
IV	intravenous	TBSP	tablespoonful (15 ml)
kg	kilogram	t.i.d.	three times daily
lb	pound	tsp	teaspoonful (5 ml)
m^2	meter squared	Ut. dict	as directed
mg	milligram		

Do not use q.d., q.o.d., and s.i.d. because they are confused with other abbreviations; s.i.d. is unknown to health professionals outside of veterinary medicine. If in doubt about an abbreviation, write out the directions in plain English. Also, avoid using abbreviations such as b.i.d., t.i.d., q.i.d., as they are confusing to clients. Abbreviations such as q12h, q8h and q6h are much clearer.

Prescription Writing

A prescription is a written order or other order, which is promptly reduced to writing for a controlled substance or for a preparation, combination, or mixture thereof, issued by a practitioner who is licensed in his state to administer and prescribe drugs in the course of his professional practice. A prescription does not include an order entered in a chart or other medical record for drugs administered. Always store blank prescription pads in a secure place with access limited to minimal personnel. The elements required in a valid prescription are provide in Tables 56.9 and 56.10. When writing a prescription, abbreviations are allowed. Some commonly accepted and recognized abbreviations are listed in Table 56.11. When in doubt about an abbreviation, *spell it out.*

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Medication Control Programs in Performance Animals

Cynthia A. Cole

Regulation of Drug and Medication Use in Performance Animals

Horses, dogs, and other species frequently compete in athletic events in which the use of drugs and medications are controlled. The programs are often referred to as doping control: doping being the colloquial term for the illegitimate use of drugs to alter athletic performance in animals and man. The goals of medication control programs generally fall into three categories: (i) assure a fair and level playing field for the competitors, (ii) protect the health and welfare of the animal and human participants, and (iii) safeguard the public interest, whenever parimutuel wagering is involved. A major challenge in these programs is to control doping, but not to interfere with the legitimate use of veterinary medications. In addition, what is a medication in one setting could be seen as doping in another. For example, medicating a horse during the training phase to assist in healing from an injury is entirely legitimate, but administration of those same medications on race day, would likely be construed as doping (i.e., trying to make a sore horse sound enough to compete). Rarely is the differentiation between doping and appropriate veterinary care so obvious, however. For example, as laboratory methodologies have improved, it is now possible to detect some drugs days, weeks, or in some cases a month or more after they have been administered. This has led to the pivotal question of what is appropriate medical treatment of animal athletes. Treating a horse or dog during training is generally deemed acceptable, but residues of those medications can be detected long after they cease to have any demonstrable pharmacological activity. The difficulty in these situations is differentiating between a pharmacologically insignificant residue and one that may be associated with a pharmacological effect, even if that effect is small.

Overview of Regulatory Agencies and Stakeholder Organizations

It is important for veterinarians who treat animals that compete in events governed by medication control programs to understand that the regulatory environment is a dynamic one. What is permitted today in a program may be forbidden tomorrow. In addition, the sensitivity of the testing methods used to regulate compliance with medication rules continues to increase. As a result, the times required to discontinue the administration of medications prior to a competition in order to avoid positive tests, often referred to as withdrawal times, are also increasing. Veterinarians need to know what regulatory agency has authority over the events in which their clients are competing, so they can keep abreast of changes in medication rules and help their clients comply with them.

Racing Animals

Although there has been some discussion among industry stakeholders of the need for federal regulation, currently, medication rules for racing animals in the United States are determined by the individual states. States may have a Racing Commission, a Division of Parimutuel Wagering, or some other state agency that is given the authority and responsibility of regulating racing animals, horses and/or Greyhounds. It is the parimutuel wagering component of racing that has resulted in government oversight of these animal sports. Rules can vary significantly from state to state, so it is important to review the regulations in each state.

Although each state sets its own rules and regulations, there are several organizations that work to encourage harmonization of the programs. This is important because over a course of several weeks or months many

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horses may routinely race in several different states. The Association of Racing Commissioner's International (ARCI) consists primarily of racing regulatory officials from the USA, Canada, and Caribbean (www.arci.com). It has no direct rule making authority, but the organization has developed model rules and penalty guidelines, which many states use as a framework for their regulations.

Another industry association is the Racing Medication and Testing Consortium (RMTC) the stated goals of which are to: develop and promote uniform rules, policies, and testing standards at the national level; coordinate research and educational programs that seek to ensure the integrity of racing and the health and welfare of racehorses and participants; and protect the interests of the racing public (http://rmtcnet.com). The organization consists of racing industry stakeholders and, similar to ARCI, it has no regulatory authority, but it does wield a great deal of influence. It has funded a significant amount of research related to drug and medication issues in racing horses and has developed a comprehensive Laboratory Code of Standards, as well as operating an External Quality Assurance Program. It also has developed withdrawal guidelines for many therapeutic substances and has worked closely with ARCI on model rules and a classification guideline for foreign substances.

In 1993 the International Federation of Horseracing Authorities (IFA), which has member countries around the world, was formed to: coordinate and harmonize the rules of the member countries regarding breeding, racing, and wagering, ensure the quality and fairness of racing, protect the welfare of horses, jockeys, and the people attending horseraces, and keep horseracing up to date with technical, social, and economic changes (www.horseracingintfed.com). Its interests are broader in scope than either the RMTC or ARCI, which are more North American centric, but its influence in the North America is also less than those other organizations.

Nonracing Drug and Medication Rules

In the United States the largest governing body for drug and medication rules covering nonracing horses is the United States Equestrian Federation (USEF). A private, nongovernmental organization, the USEF regulates the vast majority of equine nonracing athletic events in the USA, including disciples recognized by the Federation Equestre International (FEI), which represents the most elite levels of equestrian competitions worldwide. The USEF has two rules under which the various breed groups and riding discipline organizations can choose to hold their competitions. Most breeds and disciplines that compete under USEF rules are subject to the Therapeutic Substance Provisions, which allows certain therapeutic medications to be present in clinically significant concentrations, while banning the presence of others outright. The Prohibited Substance Provisions rule, under which all FEI and Endurance competitions are conducted, classifies drugs as either controlled medications or banned substances. In this rule even controlled medications can only be present in nonpharmacologically significant concentrations, and therefore it is a much stricter rule than the Therapeutic Substance Provisions rule. The exact rules and regulations are updated frequently and details on both rules and advice on how to comply with them can be found on the USEF website (https://www.usef.org/_IFrames/Drugs/Default.aspx).

The Drug Testing Process

In order to ensure compliance to drug and medication rules, biological samples are collected from animals participating in competitions either during or immediately after the event. There are a number of private and state run forensic drug testing laboratories that specialize in analyzing these samples for the presence of nonpermitted medications and overages of permitted, but controlled, medications. The testing process aims to separate and identify nonendogenous components or drugs in biological matrixes. The process can be separated into four steps: (i) sample collection, (ii) extraction, (iii) separation, and (iv) detection and identification.

Sample Type

Most commonly, urine and/or blood samples are collected and analyzed for the presence of nonpermitted substances and overages of permitted medications. Traditionally, urine has been used primarily for regulating nonpermitted substances, whereas blood samples were used to control permitted medications through adoption of regulatory limits or thresholds, but this approach is changing.

The selection of urine as the analytical matrix of choice is often made because it can be collected noninvasively with relative ease, particularly in horses. The large volumes typically voided in horses are also an advantage. In dogs, collection is a bit more difficult and volumes are often limited, but it is still the most common fluid collected in drug testing programs. Because many, if not most, drugs are eliminated in the urine and the kidney concentrates the urine, drugs can generally be detected in urine samples longer than they can be detected in blood samples. One limitation of the use of urine, however, is that it is very difficult to determine the pharmacological significance of the analytical finding, because urine concentrations will vary dramatically with the specific gravity of the urine.

Many medication control programs have been moving toward exclusively using blood samples, and the resulting serum or plasma collected from them, as the testing matrix. Even though, as previously mentioned, the concentration of drugs in the plasma/serum is often less than it is in urine, recent advances in technology have tremendously increased the sensitivity of analytical methods, generally rendering this disadvantage irrelevant. A major advantage of the use of plasma/serum harvested from blood samples is that the concentrations can more easily be correlated to pharmacodynamic effects if the relevant pharmacokinetic/pharmacodynamic studies have been conducted. A disadvantage to their use is the need for venipuncture, which is invasive and requires at the minimum a trained veterinary technician, if not a veterinarian.

Alternative matrixes, such as hair, have been investigated but their use is problematic. It would be extremely difficult to prove the time frame of exposure when a drug is found in a hair sample, and some horses may change hands several times in the course of the year. Saliva has also been used as a testing matrix, but because it is difficult to obtain sufficient volume to allow for a confirmatory analysis, its usefulness is limited.

Analytical Methods for Extraction

The goal of an extraction process is to isolate and concentrate the drug, referred to as an analyte, from the sample. One of the simplest and oldest extraction methods is a liquid–liquid extraction (LLE). In LLE a liquid biological matrix, such as urine or plasma, is mixed with an organic solvent that is not miscible with the sample. Most drugs are more soluble in the organic solvent than in the aqueous matrix of the sample, so they concentrate into the solvent during the mixing process. The solvent is separated from the aqueous phase and usually concentrated by evaporation to increase the concentration of the analyte in the final reconstitution buffer, which will increase the sensitivity of the assay. In solid-phase extraction (SPE) the organic matrix is passed through a column containing beads to which functional groups, such as hydrocarbons or ion exchange groups, have been bound. The analytes bind to the beads allowing the organic contaminants to be removed by washing the column. In the final step the column is buffered to a specific pH to facilitate release of the analytes, which are then removed from the column with a small amount of elution buffer. The buffer is generally removed by evaporation and the sample reconstituted in a solvent amendable to analysis. Analytes may also be extracted from the sample matrix by precipitation, which relies on differences in the solubility of the analytes and other compounds in the matrix. This process is commonly used to remove the proteins from plasma or serum, after which the resulting supernatant can be directly analyzed.

It is important to realize that different extraction processes may be more or less efficient at separating drug from the biological matrix. This can have significant regulatory ramifications because the final determination of the amount of drug present in the sample will depend to some extent on the extraction method used. So if two laboratories analyze the same sample using different extraction methods, the final concentrations of the analyte determined by the labs may be slightly different. The extraction efficiency is often determined during validation of an analytical method, but rarely is it factored into the determination of the final concentration in a biological sample.

Analytical Methods for Separation

The goal of the separation process is to isolate analytes from each other after extraction and prior to the detection process. The most common method used in forensic drug testing laboratories for the separation of analytes is chromatography. For a chromatographic separation the extracted sample is reconstituted in a mobile phase and passed through an immiscible stationary phase. Differences in the chemical characteristics of the analytes, such as polarity, size, and charge state, will cause them to have different rates of migration through the stationary phase. These differences, when optimized, result in the analytes moving through the column in discrete waves or bands, which can be collected or diverted to other instruments for further analysis.

One of the oldest types of chromatography is planar chromatography, where the stationary phase is bonded to a piece of glass or paper, and the mobile phase passes over it by either gravity or capillary action. Thin layer chromatography (TLC) is a type of planar chromatography that was used for many years in forensic drug testing laboratories as a primary screening test. Because it is possible for different analytes to have similar migration patterns, the findings are only presumptive and a second confirmatory method must always be employed. TLC is extremely labor intensive and not as sensitive as more modern chromatography methods, and therefore it is not used with much frequency today.

In column chromatography, the stationary phase is contained within a column and the mobile phase, either liquid (LC) or gas (GC), is passed through it by either gravity or most commonly positive pressure. In GC, samples are vaporized in a heated inlet kept in excess of 200°C and moved through the column by an inert carrier gas, such as argon, helium, or nitrogen. Typical GC columns are several meters long with an internal diameter of 1 mm or less. One of the limitations of GC analysis is that many of the analytes of interest to forensic drug testing laboratories are not stable at high temperatures. Therefore, they are often derivatized to other compounds to make them more stable and volatile. The derivatization process can be time consuming and it complicates the analysis, which is another of the drawbacks to GC methodology. GC systems are fairly straightforward to operate, however, and for some compounds GC can be a very efficient separation method.

In liquid chromatography the analytes in a sample are separated by utilizing the differences in their physiochemical characteristics as they pass through an absorptive column. Typical LC columns are 100–500 mm long with an internal diameter of 3 mm or less. In LC methods the characteristics of both the mobile and stationary phases can be modified to optimize analytical separation. Another advantage of LC analysis is that it uses aqueous mobile phases at room temperature. Most of the compounds of interest to forensic drug testing laboratories are quite amenable to separation by LC, and therefore it is the most common separation method used today.

Analytical Methods for Detection

Once the analytes have been extracted from the biological matrix and then separated from each other, they must be identified. The two most common methods used in forensic drug testing laboratories to identify analytes are absorption spectroscopy and mass spectrometry.

Absorption spectroscopy detection methods employ ultraviolet or visible light, capitalizing on the property that many molecules will absorb certain frequencies of light. The degree of absorbance is generally directly proportional to the concentration of the molecule in solution. Because most absorption spectroscopic methods are very robust and relatively inexpensive, they are commonly used for quantitative analysis. One of the limitations of absorption spectroscopy, however, is that two different analytes can have overlapping absorption spectra and similar elution times, making them difficult to differentiate. In addition, the presence of chemical and/or spectral interferences can cause erroneous detector responses resulting in quantitative errors. As such absorption spectroscopic methods are not appropriate for definitive confirmation of the identification of an analyte, but are used to confirm overages of permitted medications, which have established thresholds.

In mass spectrometry (MS), following extraction and separation, the analytes are serially injected into the mass spectrometer for identification. While many different types of mass spectrometers are commercially available, the type most commonly used in forensic drug testing laboratories bombards the analyte with a beam of electrons that causes the molecules to become ionized. While some of these ions will remain intact, referred to as precursor ions, many will fragment into smaller ions and neutral fragments. The precursor ions and the product fragments are separated in electric or magnetic fields based on their mass to charge ratio and then serially passed into a detector that measures the abundance of each ion. A plot of the abundances of the precursor and product fragment ions versus their mass to charge ratios is referred to as the compound's mass spectrum (Figure 57.1). The highest molecular weight peak in a spectrum usually represents the parent molecule minus a single electron (i.e., ionized), and is referred to as the molecular ion. The mass spectrum of the analyte is compared to a computerized database of mass spectra, referred to as a library, or to an authenticated standard of a known compound. Because the fragmentation pathways for many molecules are well characterized and extremely reproducible, unambiguous identification of a compound can be achieved using MS. Even analytes with the same nominal mass can be differentiated, because they will produce different fragmentation patterns. Because the abundance of the ions is directly proportional to the concentration of the analyte in the solution, extremely accurate quantitation is possible using MS methods. One of the disadvantages of MS is that it requires highly trained analysts to develop the methods and interpret the data. Nevertheless, because of the high degree of specificity inherent in the results, mass spectrometry has become the de facto standard method for drug identification in forensic drug testing programs.

In practice, the separation and detection processes are combined. An LC or GC instrument is placed in front of the mass spectrometer leading to the common designation of LC/MS or GC/MS. In addition, newer instrumentation can repeat the ionization and fragmentation processes on the original fragmentation ions, so-called LC/MS/MS or LC/MSn. This process can dramatically increase both the accuracy and the sensitivity of the method.

Testing Scheme Overview

Regardless of the type of sample collected (i.e., blood, urine, hair, etc.) the testing procedure must take into consideration both scientific and legal issues. An extremely important legal aspect of the process is the chain-of-custody documentation, which is a tamper-resistant paper or electronic trail indicating where the sample has been and who has had custody of it at all times. For this reason the actual drug testing process begins when the sample is collected. If there are questions about the integrity of that process, it puts the results of the final analysis in to question. For example, because many modern mass spectrometric techniques can detect quantities of drugs and medications in the picogram to femtogram range, special care must be taken by the sampling personnel to prevent contamination from environmental

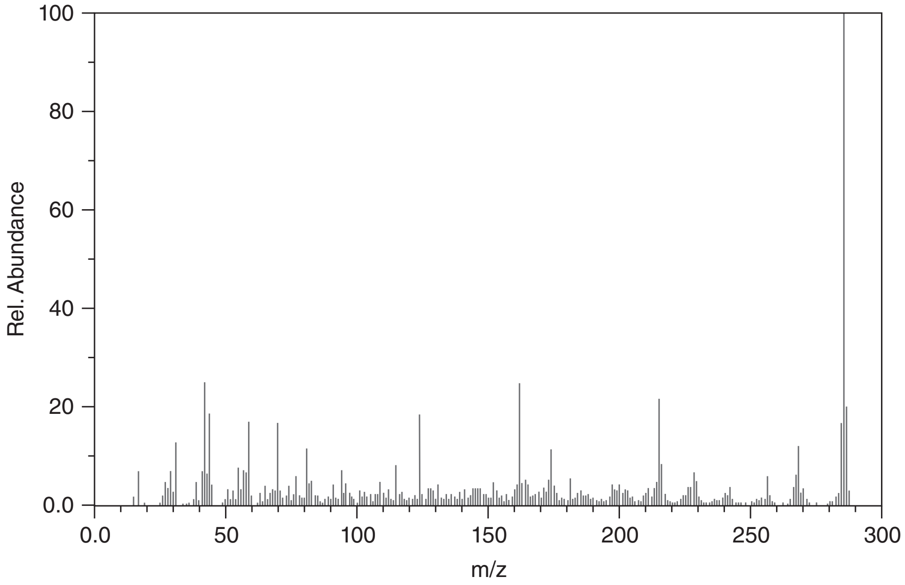

Figure 57.1 Mass spectrum of morphine. Source: National Institute of Standards and Technology.

sources during the collection process. Only clean, new, sample collection vessels should be used and personnel should wear appropriate attire, such as clean coveralls and disposable gloves. In addition, no eating, drinking, or smoking should be permitted at the collection location.

Once a sample is collected, a small aliquot is usually transferred to a separate container, which is referred to as the split, referee, or B sample, and both are sealed with tamper-proof evidence tape and secured in locked containers or coolers for transport to the testing laboratory. If the original sample is found to contain a nonpermitted substance or an overage of a permitted medication, the referee or B sample can be used to confirm the findings of the original analysis. In some cases the referee sample is sent to the laboratory with the original sample and in other cases it is stored at a separate facility. To prevent degradation, nonfrozen samples should be kept cold and shipped overnight to the laboratory. Otherwise the samples should be frozen and kept frozen throughout the shipping process.

The samples are shipped to the laboratories with a manifest that generally contains only limited information regarding the samples. Each sample will have a unique identification code and generally the manifest sheet will indicate the event or racetrack where the sample originated, the day the samples were collected, and the number of the class or race that the animal competed in that day. The laboratory does not know the name of the animal, the trainer, rider or driver, or any other identifying information regarding the animal, other than the sex in some cases. Maintaining anonymity in the testing process is crucial to ensuring its integrity and the reputation of the laboratory for fair and equitability treatment.

Once at the laboratory, the samples will generally be logged into the laboratory information management system and stored in a secure refrigerator or freezer, as appropriate, until they are analyzed. In the first step of the process all of the samples are subjected to a series of screening tests. Currently, the state of the art screening would be a combination of acid, basic, and neutral pH extractions with and without an enzyme hydrolysis step, designed to liberate conjugated metabolites, followed by LC-MS screening. Some laboratories include an enzyme-linked immunoassay (ELISA) screen and other may still use a TLC analysis, although this is becoming less common.

If any of the screening tests are positive, the sample is determined to be suspicious and sent for confirmational analysis. As a general rule, confirmation will always entail a mass spectral identification. LC/MS is the most common methodology used today in forensic drug testing laboratories, although GC/MS is also still occasionally used.

Referee Sample Analysis

As described above, before a reputable laboratory reports that a sample contains a nonpermitted substance, the sample has undergone multiple screening and confirmation tests using several different analytical methods. Nevertheless, a referee or B samples analysis is permitted in many different drug and medication rules. The referee sample is analyzed by an independent laboratory approved by the regulatory body, and, if that laboratory does not confirm the presence of the nonpermitted substance, than generally no regulatory action is taken. There are variations on how these programs are executed, so veterinarians may want to review the process with his or her client when they are discussing the applicable drug and medication rule.

Factors Affecting Withdrawal Times

A withdrawal time can be defined as the number of hours, day, weeks, etc. that should elapse following the last administration of a drug in order to avoid the animal having a detectable concentration of a nonpermitted drug in a sample collected during or after a competition. Veterinarians are familiar with withdrawal times from food animal medicine, and often want to know specific withdrawal times for therapeutic medications commonly used in animals competing in athletic events. Unfortunately, it can be difficult for laboratories to generate that information, because so many factors can affect the rates at which drugs are eliminated from the body. For example, there is often a large dose range for therapeutic medications, and the duration of treatment also varies. Some drugs, such as clenbuterol and isoxsuprine, can accumulate in the body leading to extremely long withdrawal times if the laboratory uses a sensitive analytical method. The route by which the medication is administered can also affect the withdrawal time. Drugs administered intravenously will generally be eliminated in a predictable manner, whereas drugs administered orally or subcutaneously may have more variable absorption patterns, which will in turn affect the time course of drug elimination. Laboratory factors can also affect withdrawal times because different labs may use different analytical methods with different limits of detection (LOD = the lowest concentration of an analyte the method can reliable differentiate from the background). Many of these concepts applied to food animal withdrawal times are similar and are discussed in Chapter 61.

For racing horses, the RMTC and ARCI have generated recommended withdrawal times for a number of therapeutic substances (www.rmtcnet.com) and most US forensic drug testing laboratories have agreed to follow the associated recommended detection limits. In a similar manner, the USEF has published recommended withdrawal times for medications commonly used in horses competing under their Therapeutic Substance Rule (http://www.bevet.com/files/usefmedicationinformation pdf.pdf).

Thresholds, Reporting Levels, and Cutoffs

As analytical chemistry methods have improved, the smallest amount of a drug that can be detected in a bodily fluid has progressively decreased. LODs in the picogram/ml range are not uncommon in many forensic drug testing laboratories. Because the sensitivity of the testing is so high, administration of some medications days or even weeks before a race or event can result in a positive drug test. For example, following 30 days of dosing, clenbuterol was still readily detectable for 3 weeks after the last administration (Kynch et al., 2014). In addition, contamination of the animal's diet with feedstuffs that naturally contain small amounts of prohibited substances can also be the source of positive drug tests. For example, cocoa husks, which may be used in some feeds as filler, contains theobromine, a xanthine related to caffeine and considered a prohibited substance. In a similar manner, plants in the family of *Datura* contain scopolamine and other alkaloids and may be found growing around barns and pastures.

Table 57.1 Commonly accepted reporting levels for substances with environmental sources

Arsenic	3 μg/ml in urine
Salicylic acid	625 μg/ml in urine or 5.4 μg/ml in plasma
Theobromine	2 μg/ml in urine

Regulatory authorities have in some cases have adopted concentrations for certain substances below which the laboratory will not report their presence as a violation of the drug rule. There are various names for these concentrations including thresholds, reporting levels, cutoffs, etc. There are two types of analytes for which these reporting levels are adopted. First, there are some substances that can commonly be found in the horse's food or environment, which makes it difficult for trainers to prevent their horses from coming into contact with them (Table 57.1). Second, because many therapeutic agents can now be detected for much longer than they are pharmacologically active, reporting levels have also been developed for a number of therapeutic agents by some industry stakeholder groups, such as the RMTC (Table 57.2). Veterinarians need to be aware that the reporting levels in place will vary with each regulatory authority. In addition, the adoption of these reporting levels remains controversial with some industry stakeholders, so veterinarians should be aware that the levels may vary depending on the jurisdiction and the equestrian discipline.

Current Controversies

Furosemide

Furosemide is a loop diuretic that acts by inhibiting luminal Na^+-K^+-$2Cl^-$ transporters in the thick ascending limb of the Loop of Henle. By blocking the reabsorption

Table 57.2 Racing Medication and Testing Consortium recommended reporting levels for some commonly administered therapeutic agents

Medication	Withdrawal guideline	Threshold	Route of administration	Experimental dose
Betamethasone	7 days	10 pg/ml of plasma or serum	Intraarticular	9 mg total in one articular space
Dexamethasone	72 hours	5 pg/ml of plasma or serum	Intravenous	0.05 mg/kg
Firocoxib	14 days	20 ng/ml of plasma or serum	Oral	0.1 mg/kg for 4 days
Methocarbamol	48 hours	1 ng/ml of serum or plasma	IV/Oral	15 mg/kg IV; 5g orally

of Na^+, Cl^-, and water it causes significant diuresis. In the USA and Canada, furosemide is frequently administered no less than 4 hours prior to racing to horses that suffer from exercise-induced pulmonary hemorrhage (EIPH). In the rest of the world, furosemide is considered a forbidden substance in all horses, regardless of their EIPH status.

Whether furosemide is therapeutically beneficial for horses that suffer from EIPH is extremely controversial. It was originally hypothesized that by decreasing the circulating plasma volume, furosemide might decrease the high intravascular pulmonary capillary pressure that has been proposed to be the underlying pathophysiological mechanism of EIPH (Hinchcliff and Muir, 1991). Numerous studies conducted under both laboratory and racetrack conditions have yielded conflicting results as to its efficacy in decreasing the severity of EIPH (Sweeney and Soma, 1984; Pascoe et al., 1985). A large, well-controlled study conducted on race horses in South Africa, however, did demonstrate, quite compellingly, that furosemide decreased the incidence and severity of EIPH (Hinchcliff et al., 2009). There is also substantial evidence, however, from studies conducted under both laboratory and racing conditions that furosemide can enhance athletic performance in horses (Soma and Uboh, 1998). Despite the evidence that furosemide may be of therapeutic benefit to horses that suffer from EIPH, at the present time some racing industry stakeholders have suggested that it should be a forbidden substance in US racing. The argument made is that it supports a negative stereotype of racing, where horses have to be "drugged" in order to compete and that making it a banned substance would be consistent with racing regulations worldwide.

Anabolic Steroids

Until 2009, the use of anabolic steroids in racing horses in the USA, while not technically permitted, was basically allowed because most laboratories were not analyzing postrace urine samples for their presence. This was in stark contrast to most racing jurisdictions globally, where anabolic steroids were considered forbidden substances and where laboratories judiciously tested for their presence. Following a series of dramatic catastrophic injuries in high-profile races in 2008 that resulted in a significant amount of negative press, racing jurisdictions in the USA began to adopt regulations limiting the use of anabolic steroids. It should be noted that a direct link between treatment with anabolic steroids and an increased risk of suffering a catastrophic injury has never been made. The RMTC recently released recommendations for plasma thresholds for a number of anabolic steroids commonly used in racehorses, calling for significantly prolonged withdrawal times (Table 57.3). Previous thresholds were adopted in urine, but the results of recent studies have suggested that boldenone and nandrolone can be formed ex vivo in the urine by enzymatic conversion of testosterone (Guan et al., 2012; Soma et al., 2012). The ex vivo conversion of testosterone to boldenone was confirmed in a urine sample collected from a Standardbred stallion in Australia (author's personal experience). In addition, the spontaneous conversion of testosterone to both nandrolone and boldenone has been confirmed in urine samples collected from intact cattle (Poelmans et al., 2005; Le Bizec et al., 2006; Dervilly-Pinet et al., 2011). It may be that contamination of equine urine samples with fecal bacteria is the mechanism by which the conversion

Table 57.3 Plasma thresholds for anabolic steroids recommended by the Racing Medication and Testing Consortium

Anabolic steroid	Threshold	Withdrawal recommendation
Boldenone	25 pg/ml for all horses regardless of sex	82 days
Nandrolone	25 pg/ml for geldings, fillies, and mares (male horses other than geldings will not be tested for nandrolone in blood)	35 days
Stanzolol	25 pg/ml for all horses regardless of sex	47 days
Testosterone	25 pg/ml for geldings, fillies and mares and a confirmatory limit of 2 μg/ml for male horses other than geldings, which regulates a 30-day withdrawal time	30 days

occurs, but this has not been confirmed. One of the arguments in favor of the use of plasma as the testing matrix, instead of urine, is that bacterial contamination is exceedingly rare, and therefore ex vivo formation may be less likely to occur. If a racing jurisdiction adopts the RMTC thresholds the extremely long withdrawal times make the use of anabolic steroids in racehorses in training in that jurisdiction practically impossible.

Recombinant Products

Traditional drug testing processes have been focused on small drug molecules. Since the introduction of glycoprotein and peptide hormones, such as recombinant erythropoietin (rEPO) and recombinant growth hormone (rGH), their detection and differentiation from endogenous analogs has been a challenge for forensic drug testing laboratories. These are large molecules and, depending on whether their origin is equine or human, they may be identical or almost identical to the endogenous molecule. In addition, after administration their effects on the body last far longer than current analytical methods are capable of detecting their presence in urine or blood samples.

The endogenous hormone erythropoietin, which is produced by the kidneys, regulates erythrocyte and hemoglobin production in mammals. In humans use of an erythropoietin peptide produced by recombinant technology (rEPO) has been shown to increase aerobic athletic performance by increasing the oxygen carrying capacity of the blood (Audran et al., 1999; Birkeland et al., 2000). Horses, however, possess a contractile spleen that can release up to 12 liters of blood during periods of stress, excitement, or exercise (Toutain, 2010). Because horses are in essence natural blood dopers, it has always been questioned whether rEPO administration would enhance athletic performance in equine athletes. In one study in unfit horses rEPO enhanced aerobic capacity, but did not improve athletic performance (McKeever et al., 2006). The effect of rEPO on fit horses, however, has not been determined. In addition to possible performance enhancing effects, rEPO also has been associated with the development of severe anemia in horses (Piercy et al., 1998; Woods et al., 1997). All rEPO products are derived from the human peptide sequence and it appears the small differences between the final structure of the human and equine peptide are significant enough for the body to recognize the protein as foreign. The antibodies generated against the human protein cross-react with the endogenous equine hormone, preventing it from functioning, resulting in anemia, and in some cases death. Therefore, regardless of its performance enhancing effects, or lack thereof, rEPO is generally not permitted to be administered to horses subject to medication control programs.

A great deal of effort and money has been expended to develop methods to detect the administration of rEPO to horses. In one study following IV administration of rEPO, the peptide could be readily detected in urine by ELISA for up 10 hours postdosing, but by 48 hours plasma concentrations had returned to baseline (Tay et al., 1996). Using LC/MS/MS methodology rEPO was detectable for several days following administration (Guan et al., 2007). The half-life of equine red blood cells is relatively long at 140 days, however, and therefore if there were benefits to the use of rEPO, they would last long after it could be detected by current testing methods. This situation has led to adoption of out of competition testing in many jurisdictions. A number of states have mandated that blood can be collected from any horse on racetrack grounds, regardless of whether the horse is actually entered to race. These samples can then be analyzed for the presence of rEPO.

Recombinant growth hormone (rGH) has been available in Australia for a number of years. Sold under the brand name EquiGen™ Injection, it is a freeze-dried form of methionyl equine somatotropin (eST) or equine growth hormone produced by recombinant DNA technology. In one study the athletic performance of unfit mares was not enhanced by rGH administration (McKeever et al., 1998). Regardless, it is considered a nonpermitted substance in all regulated competitions, although its detection is extremely problematic at this time.

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Pharmacovigilance

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Introduction

FDA Overview

The US Food and Drug Administration (FDA) is a regulatory, science-based federal agency responsible for protecting and promoting the public health through monitoring and regulation of a variety of products necessary for the health and well-being of consumers. The FDA's jurisdiction includes most food products (other than meat and poultry), animal feed and pet food, human and animal drugs, human dietary supplements, medical devices, veterinary devices, therapeutic agents of biological origin for humans (e.g., vaccines), radiation-emitting products for consumer, medical, and occupational use, cosmetics, and tobacco products.

FDA is an agency within the Department of Health and Human Services and is organized into five major offices under the Office of the Commissioner. The Center for Veterinary Medicine (CVM) and the Center for Food Safety and Applied Nutrition (CFSAN) report directly to FDA's Office of Foods and Veterinary Medicine (FDA, 2017a).

Center for Veterinary Medicine

Among other things, the Center for Veterinary Medicine is responsible for ensuring that animal drugs, including drugs intended for use in or on animal feed, are safe and effective for their intended uses and that food from treated animals is safe for human consumption. Before a new animal drug can be legally marketed in the United States, it must be approved by the FDA on the basis of quality, safety, and efficacy. When the drug is to be approved for use in food-producing animals, safety to the target animal species must be demonstrated, in addition to safety of food products derived from the treated animals that are intended for human consumption. Once approved products are on the market, the Center monitors the use of the products through surveillance, compliance, and pharmacovigilance programs. Currently, the Center for Veterinary Medicine is organized into six offices. Information about each office and their roles and responsibilities can be found on CVM's web site (FDA, 2017b).

CVM's Office of Surveillance and Compliance (OS&C) has primary responsibility for several of the Center's core functions, including compliance-related actions, postapproval monitoring, and animal feed safety. Within OS&C, the Division of Veterinary Product Safety (DVPS) is responsible for monitoring the safety and effectiveness of marketed animal drugs and devices, and the safety of pet food, through review and analysis of adverse experience reports. The information obtained from review and analysis of these reports helps CVM make decisions about product safety, potentially leading to regulatory actions, label revisions, or other changes necessary to ensure the safe and effective use of a product. Submitted adverse experience reports are maintained in a database used by OS&C to conduct postapproval surveillance and pharmacovigilance activities.

Overview of FDA's Postapproval Surveillance Program

Although CVM has a rigorous preapproval process for animal drugs, well-conducted randomized controlled clinical trials may not be of sufficient size to identify every safety problem. Once a product is marketed, there is a substantial increase in the number of patients exposed to the drug, including animals with coexisting medical conditions and those being treated with concomitant medications and biologic agents such as vaccines. There are potential food interactions as well. Additional information about medical product safety and effectiveness is obtained after a product is marketed and used under actual field conditions in large diverse populations of animals. This information is used to complete the safety profile of a product and helps to ensure that drug product labeling is adequate and accurate. Ultimately, this information will assist practitioners in making informed decisions to minimize risks while maximizing benefits of the drugs used in animals.

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Pharmacovigilance and Adverse Event Reporting at CVM

Pharmacovigilance

Pharmacovigilance, as defined by the World Health Organization (WHO), is "the science and activities relating to the detection, assessment, understanding, and prevention of adverse effects or any other drug-related problems" (WHO, 2006). The goal of a veterinary pharmacovigilance program is to ensure the continued safety and effectiveness of animal drugs once they are being used in a wide and diverse population of animals.

In general, the role of a pharmacovigilance program is to identify safety signals that, upon further evaluation, may lead to the discovery of previously unidentified or unrecognized adverse drug events and associated risk factors that were not identified in the preapproval evaluation of the product. These adverse events may be related to previously unrecognized pharmacological effects of the drug, idiosyncratic effects, drug–drug interactions, drug–food interactions, drug–disease interactions, factors specific to certain patient populations, individual patient factors, medication errors, product defects, or other factors, such as the reaction being too uncommon to be identified in the small number of animals in which the drug is tested in preapproval studies (CIOMS, 2010). Spontaneous reports of adverse drug experiences, medication errors, and product defects comprise the primary data source upon which CVM's postapproval pharmacovigilance efforts depend. Also, data from postapproval clinical studies and from the scientific literature may be utilized. Limitations of utilizing spontaneous adverse drug experience (ADE) reporting databases for pharmacovigilance activities include the significant underreporting of ADEs, limited detail in submitted reports, and reporting biases (Strom and Kimmel, 2006). Given the variability in reporting and the many factors that affect reporting, it is well accepted that reporting rates cannot be used to reliably estimate incidence rates of ADEs in the exposed population, and that comparison of reporting rates between products or between countries cannot be used to determine risk. Despite these limitations, the monitoring and evaluation of ADE reports is very important to help ensure that the overall balance of risks and benefits of a particular drug remain acceptable. Additionally, it allows for communication of essential drug safety information to veterinarians and others involved in the treatment of animals.

Adverse Drug Experience – Definition

An *adverse drug experience*, as currently defined by 21 CFR 514.3, is "any adverse event associated with the use of a new animal drug, whether or not considered to be drug related, and whether or not the new animal drug was used in accordance with the approved labeling (i.e., used according to label directions, or used in an extralabel manner, including but not limited to different route of administration, different species, different indications, or other than labeled dosage). Adverse drug experience includes, but is not limited to:

1) An adverse event occurring in animals in the course of the use of an animal drug product by a veterinarian or by a livestock producer or other animal owner or caretaker.
2) Failure of a new animal drug to produce its expected pharmacological or clinical effect (lack of expected effectiveness).
3) An adverse event occurring in humans from exposure during manufacture, testing, handling, or use of a new animal drug."

ADE reports submitted to CVM can involve approved or unapproved animal drugs, human drugs used to treat animals, events in humans exposed to animal drugs, and events related to devices used in animals.

Adverse Drug Experience (ADE) Reporting

CVM encourages veterinarians or animal owners who want to report an adverse drug event to an FDA-approved product in an animal or human, to contact the product manufacturer. In the USA, ADE reporting by veterinarians and consumers is voluntary. However, manufacturers and distributors of FDA-approved animal drugs may be subject to the mandatory ADE reporting requirements in 21 CFR 514.80, as discussed further below in this section. All unsolicited reports from veterinarians or consumers, received by the FDA via either the voluntary or mandatory route, are called spontaneous reports.

Consumers and veterinarians can report adverse drug events directly to CVM by downloading a fillable Form FDA 1932a from CVM's website (FDA, 2017c). This form should be printed and sent or emailed to FDA. Veterinarians and consumers can also report information to CVM by calling the CVM hotline at 1-888-FDA-VETS. In response to the inquiry, CVM can provide a Form FDA 1932a so that it can be completed by the reporter and mailed to CVM. More information about voluntary reporting of adverse events by consumers and veterinarians is available on CVM's website (FDA, 2017d). The regulations that address the spontaneous reporting obligations for manufacturers and distributors of FDA-approved animal drugs are contained in 21 CFR 514.80, "Records and reports concerning experience with new animal drugs for which an approved application is in effect."

ADE reports are classified into essentially four categories: (1) 3-day field alerts, (2) 15-day alert initial reports, (3) follow-up reports, and (4) periodic reports.

As defined in 21 CFR 514.80(b)(1), 3-day field alert reports contain information regarding product and manufacturing defects that may result in serious ADEs. Reports of serious, unexpected ADEs are submitted as 15-day alert or "expedited" reports. As required in 21 CFR 514.80(b)(2), these reports must be submitted on Form FDA 1932 to FDA by the applicant within 15 working days of first receiving the information. Follow-up reports are submitted on Form FDA 1932 by the applicant if significant new information is revealed during their investigation of ADEs that are the subject of 15-day alert reports. Reports of ADEs that are not serious and unexpected and reports of product defects that are not expected to result in serious ADEs are submitted in the periodic drug experience report, which is submitted every 6 months for the first 2 years after approval and annually thereafter.

ADE reporting to CVM has increased dramatically over the last decade. CVM received 28,825 ADE reports for the fiscal year 2004 and 91,592 for the fiscal year 2015 (Figure 58.1). Some of the reasons for the significant increase include an increase in number of drug approvals, especially for companion animals, label information providing contact numbers for drug companies, and the interest of the public in reporting perceived product problems. Wide access to the media and internet has also increased the public's general awareness about drug safety.

Approved versus Unapproved Drugs

ADE submission is required only from companies marketing FDA-approved and conditionally (FDA, 2017e) approved animal drugs. Currently, there are no requirements for submitting ADEs for unapproved animal drugs (animal drugs which have not gone through FDA's approval process) or human drugs used in animals. Exceptions to this are those drugs on FDA's Index of Legally Marketed Unapproved New Animal Drugs for Minor Species (the "Index"). These drugs are legally marketed for a specific use in certain minor species. Many approved animal drugs can be identified by the presence of a New Animal Drug Application (NADA) number on the label, or an Abbreviated New Animal Drug Application (ANADA) number for generic drugs, or a C-NADA number in the case of conditionally approved drugs, although these identifiers on labeling are not currently required by regulation. Veterinarians and/or animal owners are encouraged to report ADEs for unapproved animal drugs, including compounded products, to CVM using the Form FDA 1932a.

Animal Devices

Though no form of premarket approval is currently required for devices used in veterinary medicine, FDA does have regulatory oversight over veterinary devices and can take appropriate regulatory action if a device is misbranded, mislabeled, or adulterated. A few examples of devices commonly used in animals include suture material, certain types of bandage materials, intravenous catheters, anesthetic machines and equipment, as well as

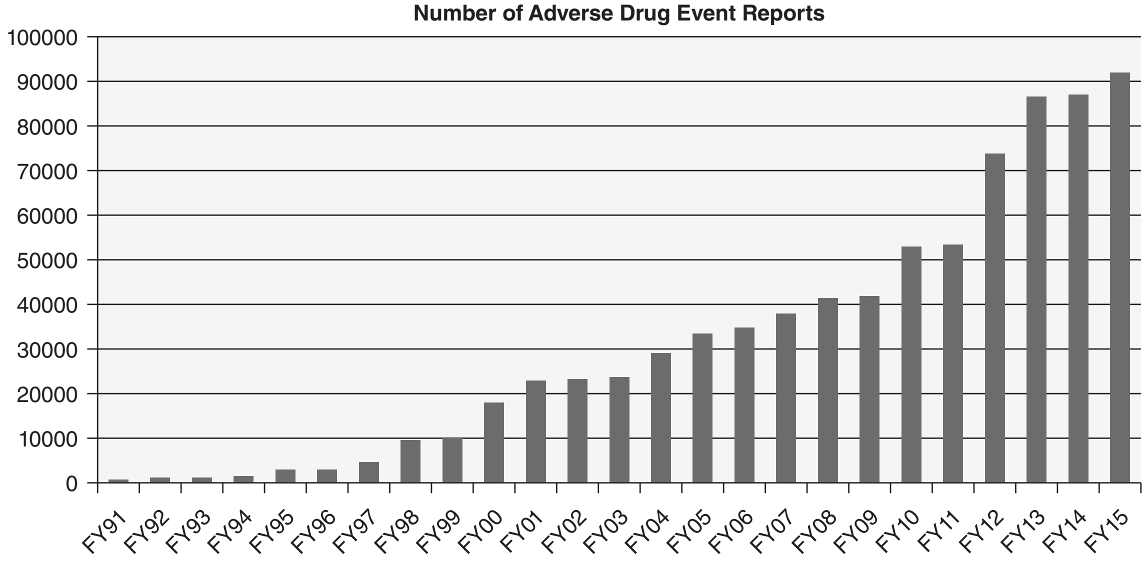

Figure 58.1 Number of adverse drug event (ADE) reports received by CVM from fiscal years (FY) 1991 through 2015.

imaging equipment. It is the responsibility of the manufacturer and/or distributor of these articles to assure that animal devices are safe, effective, and properly labeled. Although not required by regulation, CVM accepts reports from manufacturers and distributors of veterinary devices of adverse events associated with marketed devices. Most adverse event reports that CVM receives for animal devices are reported directly by veterinarians or animal owners.

Product Defects

Animal drugs, both prescription and over-the-counter (OTC), must be manufactured in accordance with Current Good Manufacturing Practices (cGMPs) according to regulation. FDA inspects manufacturing facilities before a drug application can be approved. Adherence to cGMP regulations assures the identity, strength, quality, and purity of drug products by requiring that manufacturers of drugs adequately control manufacturing operations.

Reporting Product Defects

A product defect is defined by regulation as "the deviation of a distributed product from the standards specified in the approved application, or any significant chemical, physical, or other change, or deterioration in the distributed drug product, including any microbial or chemical contamination. A manufacturing defect is a product defect caused or aggravated by a manufacturing or related process. These defects are generally associated with product contamination, product deterioration, manufacturing error, defective packaging, damage from disaster, or labeling error. For example, a labeling error may include any incident that causes a distributed product to be mistaken for, or its labeling applied to, another product." As discussed previously, reporting requirements for product defects are codified in 21 CFR 514.80. Three-day field alert reports involve product defects that may result in a serious adverse event. Product defects that do not have the potential for serious adverse events are reported directly to CVM in periodic reports. Examples of product or manufacturing defects that may result in serious adverse events include, but are not limited to, improperly labeled products, subpotent products leading to lack of effectiveness, superpotent products leading to potential toxicity, lack of sterility or particulate matter in injectable products, and failure of syringe-locking devices leading to accidental overdose of the drug (Bataller and Keller, 1999). Firms work with the appropriate FDA Field Office to accomplish corrective actions for product and manufacturing defects.

Medication Errors

The National Coordinating Council for Medication Error Reporting and Prevention (NCCMERP) defines a medication error as "any preventable event that may cause or lead to inappropriate medication use or patient harm while the medication is in the control of the health care professional, patient, or consumer. Such events may be related to professional practice, health care products, procedures, and systems, including prescribing; order communication; product labeling, packaging, and nomenclature; compounding; dispensing; distribution; administration; education; monitoring; and use."(NCCMERP, 2012). Of these, prescribing errors have been documented to cause the most harm in human patients (Strom and Kimmel, 2006). In 1992, FDA's Center for Drug Evaluation and Research (CDER) began monitoring human medication error reports that are forwarded to FDA from the United States Pharmacopeia (USP) and the Institute for Safe Medication Practices (ISMP).

In 2008, CVM began a patient safety initiative to prevent medication errors in animals. Medication errors can occur in several settings, including veterinary clinics and hospitals, universities, and human and veterinary pharmacies. Medication errors may occur for a variety of reasons, including, but not limited to:

- incomplete patient information (for example, not knowing about patients' allergies, other medicines they are taking, previous diagnoses, and lab results);
- unavailable drug information (such as lack of up-to-date warnings);
- miscommunication of drug orders, which can involve poor handwriting, confusion between drugs with similar names, misuse of zeros and decimal points, confusion of metric and other dosing units, and inappropriate abbreviations;
- lack of appropriate labeling as a drug is prepared and repackaged into smaller units;
- environmental factors, such as lighting, heat, noise, and interruptions, which can distract health professionals from their medical tasks (AHA, 2015).

Unclear medical abbreviations are one of the most common causes of medication errors.

Not all practitioners interpret abbreviations uniformly, and, therefore, the intended meaning is not always conveyed. This can occur for both written and typed prescriptions. Pharmacists may be unfamiliar with some of the medical abbreviations commonly taught in veterinary school. For example, the abbreviation "SID" (once daily) used on animal drug prescriptions is not recognized by most pharmacies and may be interpreted as "BID" (twice daily) or even "QID" (four times daily), resulting in drug overdoses for animal patients (Kim-Jung, 2010).

CVM has received several reports of medication errors of this type. Other commonly misinterpreted abbreviations include the use of “U” for units (“U” misread as zero), and the use of “μg” or “mcg” for microgram (mistaken for “mg”).

NCCMERP encourages the avoidance of abbreviations in the prescribing of medications (NCCMERP, 2005). Another common cause of medication errors is the use of trailing zeros and not using leading zeros when writing doses in medical records or on prescriptions. For example, a “5 mg” dose written with the trailing zero as “5.0” mg” can easily be misread as “50 mg.” Similarly, a “0.5 mg” dose written without the leading zero as “.5 mg” can be mistaken for “5 mg.”

FDA has worked to increase the safe use of drug products by minimizing user errors attributed to unclear nomenclature, labels, labeling, and packaging design of drug products.

Pet Food Adverse Event and Product Problem Reporting

Reporting of pet food adverse events to FDA CVM is important, and can provide an early warning of emerging food-borne diseases and problems. Additionally, owners and/or veterinarians are encouraged to contact the product manufacturer or distributor on the product label to report potential problems that may be associated with the product and/or packaging, although manufacturers are not required to forward those reports to CVM. Pet food products include pet foods, treats, puppy and kitten milk replacers, pet nutritional supplements, and pet beverages (FDA, 2017f). Examples of pet food product problems include foul odors, swollen cans or pouches, leaking containers, or foreign objects. Adverse event reports can include pet illnesses or clinical signs that an animal owner or veterinarian believes are associated with pet food product consumption. The quality of adverse event reports is enhanced if the following information is included in the report: the number of animals fed, the number that experienced the problem, the species and breed, and, if possible, the age, weight, and sex as well as the overall state of health of the animal before the adverse event. Other helpful information includes the names of any medications or other products the animal was being given or fed prior to the adverse event. The brand name, package type, package size, UPC (Universal Product Code), lot number, and use by date (found on the product packaging) as well as name and address of the manufacturer or distributor of the product and name of store where purchased is helpful information to report. Additional useful information includes the length of time between feeding the suspect food and clinical sign(s) occurrence and the amount of food consumed. The FDA website gives more information about how to report a pet food problem (FDA, 2017g).

The FDA receives reports related to pet food adverse events and product problems primarily through two channels: the Safety Reporting Portal (SRP), and the FDA District Offices. Both are components of the Pet Food Early Warning and Surveillance System (PFEWSS), which was created after the passage of the Food and Drug Administration Amendments Act (FDAAA) of 2007 in response to the melamine and cyanuric acid contamination of pet food that led to the largest recall in history of pet food in the United States. During the initial weeks of this crisis, FDA received over 11,000 pet food complaints. The combination of melamine and cyanuric acid contamination led to the development of crystals within the kidneys of dogs and cats consuming the adulterated pet foods, leading to renal failure (Dobson et al., 2008).

As part of fulfilling the requirements for a PFEWSS, the Safety Reporting Portal (SRP) was developed and went online in May of 2010 (www.safetyreporting.hhs.gov). Animal owners, concerned citizens, and veterinarians can use the portal’s pet food questionnaire to report pet food-related problems directly to CVM. Medical records, laboratory reports, photographs, and other documents can be uploaded with the SRP pet food report, and provide valuable medical information. Voluntary pet food reports are known as PFRs (Pet Food Reports). In 2014, the Livestock Food Reporting portion of the SRP opened. Consumers and veterinarians can report adverse events and product problems related to livestock feeds through this section of the portal, which is also accessed at www.safetyreporting.hhs.gov. These reports are known as LFRs (Livestock Food Reports). Another part of the SRP is the Reportable Food Registry (RFR) (www.fda.gov/Food/ComplianceEnforcement/RFR/default.htm) for mandatory reporting of specific types of product problems by manufacturers. RFR reports are submitted by manufacturers when there is a reasonable probability that an article of food will cause serious adverse health consequences. The requirements for RFR reporting are prescribed by the FDAAA of 2007.

An example of a problem that was identified through adverse event reporting using the Safety Reporting Portal is thiamine deficiency in a brand of canned cat food. A cat was diagnosed with thiamine deficiency after strictly eating one brand of canned cat food. The veterinary neurologists reported to CVM using the SRP soon after it opened in 2010, leading to a prompt product recall by the manufacturer (FDA, 2017f).

Another route that is available for reporting of pet or livestock food adverse events or product problems by owners and veterinarians is to call the FDA Consumer Complaint Coordinator that serves the geographic region in which the reporter lives. A list of the FDA Consumer Complaint Coordinators is available on the FDA website (FDA, 2017h). Early detection of pet food and animal feed problems is facilitated by reporting to the

FDA through these channels, and can lead to faster intervention by the FDA and manufacturers.

Reporting of Adverse Events associated with pesticides or vaccines

Adverse events in animals may occur in association with the administration of pesticide products or vaccines. Veterinarians can report pesticide related adverse events online to the Environmental Protection Agency (EPA) and the National Pesticide Information Center (NPIC) (http://pi.ace.orst.edu/vetrep/). FDA approves some flea and tick products as animal drugs while the Environmental Protection Agency (EPA) registers others as pesticides. This is further discussed in Chapter 43 of this textbook. The regulating agency can be identified on a product's label (FDA, 2017i). Consumers can report adverse events associated with pesticides by calling 1-800-858-7378**;** or by email at npic@ace.orst.edu. Animal vaccine-related adverse events can be reported to the vaccine manufacturer or to the Center for Veterinary Biologics at the USDA (USDA APHIS, 2011).

Evaluation of Adverse Drug Experience Reports

CVM's ADE Review Process

CVM receives ADE reports in both paper and electronic format. Voluntary reports are received by the FDA on paper or by email (Form FDA 1932a). Mandatory ADE reports (from manufacturers) submitted electronically are transmitted via the Electronic Submissions System (ESS), and the Rational Questionnaire (RQ) in the SRP (FDA, 2017j). The ESS is integrated with the FDA Electronic Submissions Gateway (ESG) allowing gateway-to-gateway submission of individual or batched reports from manufacturers. Individual mandatory reports can also be submitted by manufacturers using the SRP rather than the gateway. However, at this time, the SRP is not configured to accept adverse drug event reports from veterinarians or consumers. All reports, mandatory and voluntary, enter the CVM ADE database for analysis and archiving.

As previously described, preapproval data is limited in terms of the number of animals in which the product is evaluated, so new safety concerns can emerge once recently approved drugs enter the marketplace and are used in thousands of animals. In consideration of the large volume and complexity of ADE data which is received, CVM utilizes a triage system and focuses on recently approved drugs to complete the safety profile of the product. These products are assigned to individual Safety Reviewers in the Division of Veterinary Product Safety (DVPS) so that a specific reviewer is responsible for a particular product for its first years on the market, allowing one reviewer to monitor it closely. For recently marketed products, CVM focuses on the medical review and causality assessment of individual case reports to identify possible safety concerns.

As part of the review process, reported signs are coded and/or verified and edited as appropriate. Clinical sign coding is accomplished using the Veterinary Medical Dictionary for Drug Regulatory Authorities (VeDDRA)(EMA, 2011). The VeDDRA dictionary has a hierarchical coding schema, from the System Organ Class (SOC) down to the High Level Term (HLT), Preferred Term (PT), and Low Level Term (LLT). This allows searching for groups of signs at a more or less specific level.

Regarding causality assessment, CVM uses the Modified Kramer algorithm to weigh the possible relatedness of an adverse event to the product administered according to a set of criteria. Each clinical sign in the report is evaluated by use of this algorithm and assigned a numerical causality score, which corresponds to categories such as "unlikely", "possibly", or "probably" drug related (Kramer et al., 1979). Causality assessment methods utilizing algorithms can help to provide a more structured and consistent approach to the assessments. Due to the high volume of ADE reports submitted, CVM cannot always assess each individual report for causality. Causality assessments are generally performed on the clinical signs in ADE reports for recently approved products, and on reports that contribute to a detected safety signal under investigation (see Section Signal Detection at CVM). An alternative system, the ABON algorithm, gives an overall qualitative score to the entire adverse event report, rather than each clinical sign. The ABON system is used by the European Medicines Agency (EMA) as well as some pharmaceutical companies for causality assessment (EMA, 2004). Both causality scoring systems take into account the association in time between product administration and onset of the clinical sign, dechallenge and rechallenge with the product if this occurred, previous association of the product with the type of adverse event reported, dose relatedness, and presence or absence of an alternative etiological candidate, among other information, including whether or not there is enough information to assess the reaction.

Signal Detection at CVM

A safety signal is defined by the World Health Organization as "reported information on a possible causal relationship between an adverse event and a drug, the relationship being unknown or incompletely documented previously"(WHO, 2015). With the large volume of data CVM receives annually, safety reviewers cannot always review the reports individually. Accordingly, data mining strategies, which involve the use of computer algorithms to analyze data in large complex databases, are being employed at CVM to more efficiently identify safety signals. These methods do not replace hands-on clinical review of the case reports that generated the signal and assessment of those cases to determine the medical implications of the signal. Signals that are detected are essentially hypotheses, not causal associations, and they need to be confirmed by case series examination. These measures assist in detecting signals in large volumes of data, helping to focus time and resources.

Understanding the case series concept starts with the recognition that the ADE database provides a source of observational data for a large/diverse population. This information is used to develop a case series, which is a summary of descriptive clinical information that can be used to help characterize the drug's safety profile and identify potential risk factors associated with certain adverse events. The FDA CDER/CBER Guidance for Industry, *Good Pharmacovigilance Practices and Pharmacoepidemiologic Assessment*, provides a detailed discussion of this concept (FDA, 2005).

A CVM case series commonly includes an analysis of the following:

- the clinical and laboratory manifestations and course of the event;
- demographic characteristics of patients with events (e.g., age, breed, sex);
- exposure duration;
- time from initiation of product exposure to the adverse event;
- doses used in cases, including labeled doses, greater than labeled doses, and overdoses;
- use of concomitant medications;
- presence of comorbid conditions, particularly those known to cause the adverse event, such as underlying hepatic or renal impairment.

This guidance also includes useful information about the principles of pharmacoepidemiological assessments and factors that are typically included in causality assessments, many of which also apply to veterinary pharmacovigilance programs. Spontaneously reported data is prone to numerous biases, most important being reporting bias. A new drug with greater publicity tends to generate more reports than older counterparts (Weber, 1984). Media attention to any reported problems tend to generate more reports. Underreporting occurs as well and, in general, reporting varies over time. Some factors that may affect reporting include the severity of the reaction and the length of time the product has been marketed. Confounding can also be a problem, by concomitant products administered, by concomitant medical conditions, or by product indication. This is why individual case series review of an identified signal is very important. The quality of the ADE reports is also critical for appropriate evaluation of these potential signals. Good case reports include a detailed description of the adverse event, including time to onset of signs, concomitant drugs or other products administered (including supplements and vaccines), concurrent medical conditions, patient signalment, clinical course of the adverse event, diagnostic results and therapeutic measures, information about dechallenge and rechallenge, and patient outcome. Also, good-quality case reports include complete information about the suspect product involved, including the trade name and established name of the drug, dosage form and strength, concentration, route of administration, and lot number. For medication error reports, case reports may include information about the type of error, type of personnel involved with the error, work environment in which the error occurred, and other possible contributing factors.

If a safety signal is identified during the ADE review process, a medical review and causality assessment of the case reports generating the signal is completed. For more recently approved products, part of this process is to perform a data review, which includes a list of clinical signs for a particular product in decreasing order of their reporting frequency. The clinical sign profile that is seen postapproval is compared with the labeled adverse reactions for the product, and the development of a Postapproval Experience (PAE) section may be proposed for addition to the drug labeling. Generally, a reviewer considers both the frequency and severity of signs for PAE section development, but a specific frequency threshold is not used to determine inclusion in the PAE section. A list of potential signs for inclusion is developed for consideration. For products that already have a PAE section as part of their labeling, new signs may be added as the result of signal detection and case series analysis. Signs that are being considered for addition to a PAE section are presented to the Monitored Adverse Reaction Committee (MARC) meeting. The MARC meeting is an interactive cross-CVM pharmacovigilance forum and is held on a regularly scheduled basis. The group consists of CVM veterinarians and other scientists, who work predominantly in the CVM Office of Surveillance and Compliance and the Office of New Animal Drug Evaluation (both post and preapproval areas of the Center). During the MARC meeting, safety reviewers present the case series information they have developed and the supporting pharmacovigilance data regarding the safety signal(s) of interest. Furthermore, they participate in discussions to reach a consensus regarding the development of appropriate label revisions and Center risk management responses. In addition, pharmacology overviews of recently approved products are presented to the MARC meeting attendees by the Office of New Animal Drug Evaluation (ONADE) scientific review staff so that safety reviewers can become more familiar with the safety and effectiveness profile of new products before they are marketed.

Communication of Drug Safety Information and Regulatory Intervention

CVM's pharmacovigilance efforts contribute to the following:

- Label revisions – PAE sections, warnings, formulation changes, product packaging
- Dear Doctor letters
- Client Information Sheets (CIS)
- Responses to Freedom of Information (FOIA) requests
- Postapproval risk management programs
- Journal articles
- CVM Updates (website) and other public communications.

Communication of Drug Safety Information

The labeling of an approved animal drug product is the primary source of information about the drug, including directions for safe and effective use, contraindications, warnings, precautions, and adverse reactions. The drug labeling is evaluated as part of the preapproval process to ensure that it is scientifically accurate and provides clear instructions to veterinarians for prescription drugs and to consumers for over-the-counter animal drugs. Label revisions may become necessary after the product has been marketed and used in a large and diverse population of animals. As discussed in Section Signal Detection at CVM, addition of a PAE section to the labeling serves the important purpose of completing the drug's safety profile. Dear Doctor Letters are letters drafted by drug manufacturers and sent to veterinarians in order to notify them of important new safety information discovered after a product has been introduced to the market. This information may include any new warnings, other safety information, or other important changes to the prescribing information of a drug product. Dear Doctor Letters are often issued by the company in conjunction with label changes, although not all labeling changes result in such a letter. Dear Doctor Letters issued since 2000 are posted on CVM's web site (FDA, 2011).

For some prescription animal drugs that may pose a serious and significant risk to the health of the animals being treated or to the humans handling the animal or the drug, the FDA may require sponsors to provide additional labeling, such as a Client Information Sheet (CIS) (FDA, 2017k). These information sheets are intended for distribution by the veterinarian to the client at the time of prescribing or dispensing of the drug. Some circumstances in which a CIS may be needed for a prescription animal drug include, but are not limited to: a drug with a narrow margin of safety; a drug that may cause adverse reactions that would necessitate prompt attention; a drug that has unusual or complicated dosing instructions; or a drug with user safety issues. An example of Client Information Sheets required for a specific drug class is that for the nonsteroidal antiinflammatory drugs (NSAIDs).

CVM utilizes its web site to disseminate information about adverse drug experiences and important announcements regarding animal drug product safety. One section of the web site, "CVM Updates", contains brief press releases issued by the Center for Veterinary Medicine on developments of interest to stakeholders and the public. CVM Updates may relate to any topic, but are often used to convey information about drug safety issues.

CVM is planning to publish the Center's adverse event data on the openFDA web site (FDA, 2017). Other information and reports from the ADE database can be requested on an individual basis by filing a Freedom of Information request (FDA, 2017m). CVM scientists may also disseminate new drug safety information through publications in professional journals and presentations at professional meetings.

Safety Measures Beyond Labeling

Additional risk mitigation strategies may at times be necessary to increase the safe and effective use of animal drugs. These may include methods of communication about drug safety information as described above (changes in labeling, issuance of "Dear Doctor" letters, drug name or packaging changes), as well as other types of measures such as restricted use or distribution of a drug, or, rarely, withdrawal of a medical product from the market. Two types of special postapproval surveillance programs are Postapproval Monitoring Programs (PAMPs) and Risk Minimization Action Plans (RiskMAPs). PAMPs may include specific postmarketing study commitments which are agreed to by a drug sponsor, and are conducted after FDA has approved a product for marketing. Postmarketing study commitments are currently conducted much more commonly for human drugs and biologics, as only a few postmarketing studies for animal drugs have been conducted at this time (e.g., sometribove zinc suspension, ractopamine). A RiskMAP is a strategic safety program designed to meet specific goals and objectives in minimizing known risks of a product while preserving its benefits.

Other Surveillance Methods

Though CVM was exempt from the Sentinel Initiative, other FDA centers were tasked to establish an active postmarket risk management system. With that understanding, Sentinel's goal is to develop a national electronic safety monitoring system comprised of active surveillance methodologies related to safety signal detection, strengthening, and validation. Access to existing electronic health-care databases is critical to the Sentinel Initiative.

An important concept is that Sentinel will augment but not replace existing postapproval surveillance systems. The potential capabilities of Sentinel are impressive in that they are focused on identifying and evaluating safety issues near real time, enhancing FDA's ability to evaluate safety issues not easily evaluated with the passive surveillance systems currently in place.

Although CVM was exempt from the Sentinel Initiative, the use of sentinel surveillance methods are of interest in the field of veterinary pharmacovigilance. CVM completed a project investigating the current electronic data sources that exist in the field of veterinary medicine. The project report, *Evaluation of Potential Data Sources for Animal Drugs Used in Veterinary Medicine*, can be found online (FDA, 2017n). The scope of the project was the identification, description, and evaluation of potential data sources and/or data environments for veterinary medicine. The following elements were considered in the project:

- utility for postmarket surveillance of FDA-regulated animal drugs;
- scope, content, structure, quality, and timeliness of data;
- availability, experience, and interest of investigators with knowledge of the data in using it for postmarketing product safety surveillance as well as plans for further data source enhancements;
- barriers that exist to including each data source in the Sentinel Initiative.

Of the data sources examined, only one was designed specifically for the primary purpose of researching animal health status, diseases, and the effectiveness of veterinary medical treatments. A key finding of the project was that for postapproval surveillance to be conducted as envisioned by the Sentinel System, animal health databases would only be useful if a link can be demonstrated between the drug, the animal to which it was administered, and the animal's health status after drug administration.

CVM's International Pharmacovigilance Efforts

CVM is actively involved in international pharmacovigilance activities through its participation in the VICH program. VICH is a trilateral program concerned with developing harmonized technical requirements for the approval of veterinary medical products (drugs and biologics) in the European Union, Japan, and the United States, and includes input from regulatory and industry representatives. Canada, Australia, and New Zealand participate as active observers. The VICH program was officially developed in April 1996 and has the following full title: International Cooperation on Harmonization of Technical Requirements for Registration of Veterinary Medicinal Products.

Conclusion

The success and benefit of any pharmacovigilance system requires the constant vigilance and cooperation of all stakeholders – veterinarians, industry, academia, animal owners, and state and federal government agencies. During product development, it is not possible to identify all safety concerns. Once a product is marketed, there is generally a large increase in the number of animals exposed to the product, including those with comorbid conditions and those being treated with concomitant medical products. Therefore, postmarketing adverse event data collection and safety evaluation based on observational data is critical for evaluating and characterizing a product's safety profile and for making informed decisions on risk management. The veterinary profession and animal patients ultimately benefit from the communication of adverse drug experience information that is evaluated by CVM.

References

Note. This chapter has extensive references to current regulations. All United States Code of Federal Regulations (US CFR) are available at http://ecfr.gov. In addition, all Food and Drug Administration (FDA) website linkages are cited as FDA 2017"x" and were current as of the date that this chapter was written.

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59

Dosage Forms And Veterinary Feed Directives

Geof Smith and Jim E. Riviere

One of the primary differences between human and veterinary pharmacology is the wide range of dosage forms available to the veterinarian that occurs as a direct consequence of significant differences in the way drugs are administered to animals and humans. These differences are a consequence of obvious dissimilarity in anatomy and physiology, but also are a direct consequence of variations in behavior, husbandry practices, and inability to verbally communicate with animal versus human patients. In addition, some animals are raised for food production and thus drug delivery strategies that result in injection-site residues may not be acceptable solely due to possible human consumption of the delivery device or its residues. Different dosage forms are employed for four main reasons: ease of administration and thus compliance, controlled rate of drug delivery, ability to minimize meat or milk withholding times, and husbandry constraints in treating populations of animals in a production environment. The focus of this chapter is to introduce the reader to the wide variety of dosage forms encountered in veterinary medicine. Specifics on most of these formulations can be found in the individual therapeutic chapters or the introductory chapters on pharmacokinetics and drug absorption, distribution, metabolism, and excretion.

Pharmacokinetic Considerations and Controlled-Drug Delivery

By far, the dosage form has the greatest influence on the rate and extent of absorption of drugs. The physiology behind these factors is discussed in Chapter 2, their resulting effects on a drug's plasma concentration-time profile are presented in Chapter 3, and pharmaceutical aspects in Chapter 5. The development and impact of these formulations has been extensively reviewed elsewhere (Hardee and Baggot, 1998; Baggot, 2002; Martinez et al., 2002). When the absorption characteristics of a drug's formulation become rate limiting, and flip-flop pharmacokinetics becomes operative, the shape of the drug's concentration–time profile, and thus pharmacological effect, is in control of the formulation and not the animal's ability to clear the drug. In addition, innovations in drug delivery allow existing drugs to be more effectively used and provide extension of the commercial life of the product for a manufacturer.

The classic example of the effect of formulation is seen with the plasma concentration–time profiles of potassium, procaine, and benzathine penicillin G (Figure 59.1). The formulation strategy is to complex the active drug (e.g., penicillin G) with a moiety that delays its release to the surrounding capillary beds by modulating the drug's solubility. A pharmaceutics text should be consulted for the chemistry of these processes. The result is that the rate of release of the compound from the dosing formulation becomes slower than that of the drug's elimination, making it rate limiting.

The potential problems with these strategies are twofold. If one considers antimicrobial therapy for bacteria with very high therapeutic thresholds (e.g., minimum inhibitory concentrations, MICs), the prolonged-release formulations may never provide effective therapeutic drug concentrations. Second, such so-called "depot" preparations in food animals may result in persistent drug concentrations in tissues, thereby prolonging the withdrawal time. Drug depots at injection sites may persist much longer than effective blood concentrations and may be easily detected at slaughter. One must exhibit care to differentiate drug tissue concentrations at injection sites from those achieved after absorption and systemic distribution (Sanquer et al., 2006).

This scenario also nicely illustrates the reason that knowledge of both the extent and rate of drug absorption are needed to adequately describe the absorption of a drug and underscores why determination of drug bioequivalence (e.g., dosage form interchangeability) requires assessing both metrics related to rate (peak, time to peak) and extent (AUC) of drug absorption. The importance of these factors was also discussed in Chapter 3 under dosage regimen construction.

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Companion Website: www.wiley.com/go/riviere/pharmacology

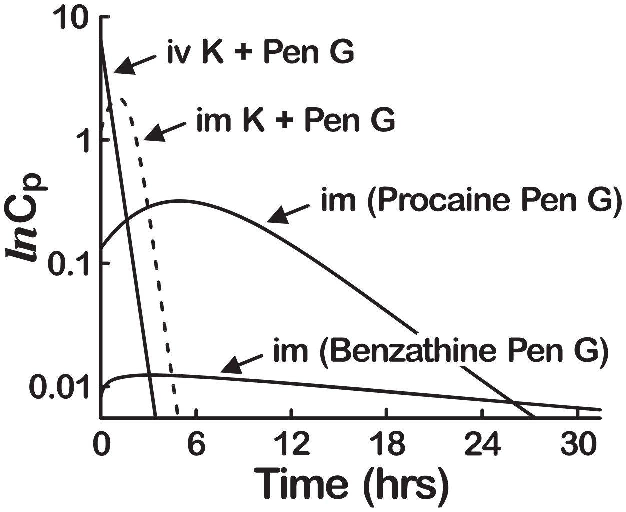


Figure 59.1 Effects of formulation and route of administration on the plasma concentration versus time profiles of penicillin G.

Approved dosages are very dependent upon the formulation of the drug utilized, a factor that must be considered when using any drug in a veterinary patient. This approach has recently been taken advantage of to develop other once-daily dosing of drugs such as antibiotics in veterinary species, the increased dosing interval being due either to the use of drugs with inherently long half-lives or depot release characteristics. These formulations stress the necessity of knowing the dosage form used when conducting any pharmacokinetic analysis as it may modulate the rate-controlling factor in drug disposition. In many cases, this can be detected only from intravenous pharmacokinetic experiments.

The development of depot preparations has received a great deal of attention by pharmaceutical industries. Some contraceptives in humans achieve monthly dosing intervals through injection in the subcutaneous tissue of insoluble tablets, which results in very slow drug release; the best example is the use of levonorgestrel implanted capsules (Norplant®). Similar strategies have been employed in veterinary medicine for the administration of growth promotants. These include estradiol formulated in rubber implants (Compudose®), progesterone and estradiol pellets (Synovex®), and zeranol (Ralgro®). Newer approaches include use of biodegradable gels that may be injected as a liquid through a syringe; these gels form a slowly degrading solid once in the tissue. Temperature-sensitive thermogels can also be used to control release of drug once in the body at physiological temperatures. A great deal of new technology is being developed for such formulations that promises more sophisticated dosage forms in the future. These approaches are fully discussed in Chapter 5.

Oral drug dosage forms achieve control of release through modulation of the surface area of dissolving drug particles, through the size of particles that alter their gastrointestinal transit time, or through the use of multiple layers in the formulation that control the rate and extent of drug release and dissolution. The simplest example is to coat a drug to prevent its dissolution in the acid environment of the stomach. These formulations are specifically designed for specific species, due to differences in gastrointestinal content and motility, as well as issues that include palatability. Capsules can be designed that initially release a bolus of readily soluble drug that functions as a loading dose into the animal. This is then followed by slower release of drug that maintains effective levels. For some compounds, only local delivery is needed to the gastrointestinal tract (e.g., certain antiparasitics), and the formulation can be designed for such activity. One recent example of this is the anthelmintic eprinomectin, which has been released in an extended therapy formulation (LongRange®) that maintains therapeutic plasma concentrations for more than 100 days. Work has occurred to specifically deliver drug to the colon in humans for localized effect. Again, it must be stressed that many of these controlled-release dosage formulations are specifically designed for species-specific anatomical (size of pyloris), biochemical (cellulose based for nonruminants), and/or physiological (pH, transit time) factors that prevent easy use in other species.

A truly unique aspect of dosage form not seen in human medicine are those employed in ruminants that take advantage of the observation that heavy materials sink in the rumen and are not passed further along the digestive tract. This has resulted in the design of mechanical dosage devices that infuse drug into the rumen for specific durations. For example a monensin controlled-release capsule is available that consists of a plastic capsule equipped with retaining wings to prevent the bolus from passing into the reticulum. The bolus sits in the rumen for an extended period of time, releasing monensin from one end for up to 100 days. Finally, slow-release osmotic pumps may be implanted in animals to achieve controlled drug infusions.

A major variable concerning parenteral injections relates to the physiology of the injection site. For a drug to be adequately absorbed from a depot preparation, there must be access to the perfusing capillaries and an adequate rate of tissue perfusion. A major source of variability is the muscle into which intramuscular injections are made. Studies have elegantly demonstrated in horses that if the injection is made between the fascial tissue bundles of a muscle group, less systemic absorption will occur than if the injection is in the muscle mass. Similarly, if the muscle group, or more likely, the SC injection site, has poor blood perfusion, less absorption may occur. If the injection results in a local tissue reaction, subsequent inflammation and fibrosis may "wall off" the drug formulation preventing absorption. Changes in ambient temperature, with compensatory changes in skin blood perfusion, may modulate absorption rate. There are numerous variables in these processes, and it is often only through the use of careful pharmacokinetic analyses that their influence on drug absorption cause can be ascertained.

Topical dosage forms include gels and patches that control diffusion from the dosage form to be rate limiting for dermal absorption. These products have been discussed in Chapter 2 and are fully reviewed elsewhere (Riviere and Papich, 2001). The advantage of dermal over oral or parenteral controlled-released approaches is that the topical gel or patch can be removed should cessation of dosing be required. Transdermal fentanyl patches for analgesia in dogs has been a particularly successful story using human patches. However, these cannot be easily transferred between species as the rate-limiting barrier may be species- and site-specific. As of this time, specific patches for animals have not been marketed.

In contrast, there are numerous topical spot-on, pour-on, collars, dip, and shampoo formulations for topical pesticides, often focusing on flea control. Some of these have resulted in once-monthly dose administration and increased owner compliance. These preparations are fully covered in Chapters 43 and 47 of the present text. In addition to these unique pour-on applications, large-animal veterinary medicine also employs ear-tags and dust-bags that allow topical drug delivery in cattle and swine in a rate- and dose-controlled fashion. Topical administration of drugs is now being expanded beyond ectoparasiticides as a novel formulation of flunixin has been developed for transdermal use in horses and cattle (Finadyne® Transdermal). The product has a longer duration of activity as compared to IV formulations of flunixin and maintains therapeutic concentrations for a greater length of time.

Palatability and Ease Of Drug Administration

Another factor important to veterinary medicine relates to the ease of drug administration to animals that might present danger to the individual administering the drug. This has resulted in formulating drugs in dosage vehicles that are palatable and will be easily eaten by the animal, but made of ingredients that do not alter drug absorption properties. A great deal of information is available from the pet food and vitamin industry that provides quantitative approaches to assess factors such as taste preferences and palatability (Ahmed and Kasrarian, 2002; Thombre, 2004). This has resulted in the development of chewable drug formulations as well as flavor ingredients that mask bitter taste or odors that animals refuse to eat. In contrast to human medicine, where sight and verbal cues may allow administering distasteful drugs to adults, animals rely on senses of taste and smell to determine whether a drug-containing formulation will be consumed. Other approaches to oral drug administration under consideration include use of dissolvable films and oral sprays for targeting the buccal cavity of patients. These are particularly being developed for cats where owners often have difficulty in administering other oral drug formulations.

A final approach that is relatively unique to veterinary medicine includes the direct administration of drug into drinking water, into lick-tanks, or on feed for use in large production settings (e.g., cattle, swine, poultry). An important distinction with this continuous form of drug administration is that, unlike the bolus effect seen with individual liquid dosing or rapidly dissolving tablets, absorption may not be first-order (Mason et al., 2009). If administration is constant in water or feed, absorption may be zero-order and have pharmacokinetic behavior resembling an infusion. Finally, drugs may be administered in medicated blocks, which results in ease of administration but little control of individual animal dosage.

Veterinary Feed Additives

One strategy for administering drugs to animals that is unique to veterinary medicine is oral dosing via feed. This is often necessary due to the need to treat a large number of animals in a production environment. Unlike individual animal dosing, this approach does not assure that a specific dose of drug reaches each animal, since drug dosage is now a function of food consumption. As mentioned above, issues of palatability and odor also must be taken into consideration.

The legal control of feed additives is also different than other drug formulations. The Veterinary Feed Directive (VFD) refers to a relatively new category of medicated feeds created by the Animal Drug Availability Act of 1996. Prior to this time, the Food and Drug Administration (FDA) had only two options for regulating the distribution of animal drugs: over-the-counter or prescription. The primary purpose of the VFD was to provide an alternative to these categories for certain therapeutic drugs that are mixed in feed. A VFD drug still ensures the participation of a veterinarian, but it allows producers to acquire medicated feeds through traditional manufacturers without involving a pharmacist or having to follow state pharmacy laws.

In 1996, the Center for Veterinary Medicine (CVM) of the FDA expressed a greater need for control over the use of certain antimicrobial agents in medicated feeds. Initially, it was suggested to mandate all new therapeutic agents approved for use in feed be for use by prescription only. However, having additive drugs approved as prescription-only was unacceptable to the animal agriculture and feed industry. Requiring these drugs to be for use by prescription only would have turned feed mills into drug stores because they would be forced to mix a new batch of feed each time a prescription was written. Studies done by the American Feed Industry Association (AFIA) in the 1990s estimated the average feed costs in the swine industry would have risen from $36 to $100 per ton because of additional costs associated with special ordering and the need to mix only small volumes of feed at once. These prescription drugs would also fall under the direction of most state pharmacy regulations, which would further complicate the ability to manufacture and distribute medicated feed.

The VFD was therefore created to be an alternative to the traditional prescription classification. It places a much greater emphasis on professional control as compared to over-the-counter medications while still allowing agricultural producers to obtain medicated feeds efficiently and cost effectively. In 2014 the FDA published Guidance 213 to limit the use of feed and water antibiotics in food animals to “uses that include veterinary oversight or consultation.” Therefore beginning in 2017, all approved feed grade antibiotics used in food animal production require VFD approval prior to use. Over the counter feed or water medications have been phased out as well as label indications that would be considered non-therapeutic (for example using antibiotics to promote feed efficiency). As with other medicated feeds, the extralabel use of any VFD drugs is prohibited. The lone exception to the above rule are ionophores such as monensin or lasalocid which are not considered “medically important antibiotics.” Chapter 55 should also be consulted for further discussion of drug regulations.

The initial requirement for using a VFD medicated feed is a valid veterinarian-client-patient relationship. As defined by the American Veterinary Medical Association (AVMA), this relationship exists when:

1) The veterinarian has the responsibility for making medical judgment regarding the health of the animal(s) and the need for medical treatment, and the client has agreed to follow the instructions of the veterinarian.
2) There is sufficient knowledge of the animal(s) by the veterinarian to initiate at least a general or preliminary diagnosis of the medical condition of the animal(s). This means the veterinarian has recently seen and is personally acquainted with the keeping and care of all the animal(s), and/or by medically appropriate and timely visits to the premises where the animals are kept.
3) The practicing veterinarian is readily available, or has arranged for emergency coverage, for follow-up in case of adverse reactions or failure of the therapeutic regimen to solve the problem.

Once a veterinarian has made an initial diagnosis, he/she can issue a signed VFD on a preprinted, multipart form. The veterinarian gives the form to the producer, who then orders the medicated feed from the feed supplier. A VFD feed may not be distributed to a producer without a signed VFD form. Three groups are involved:

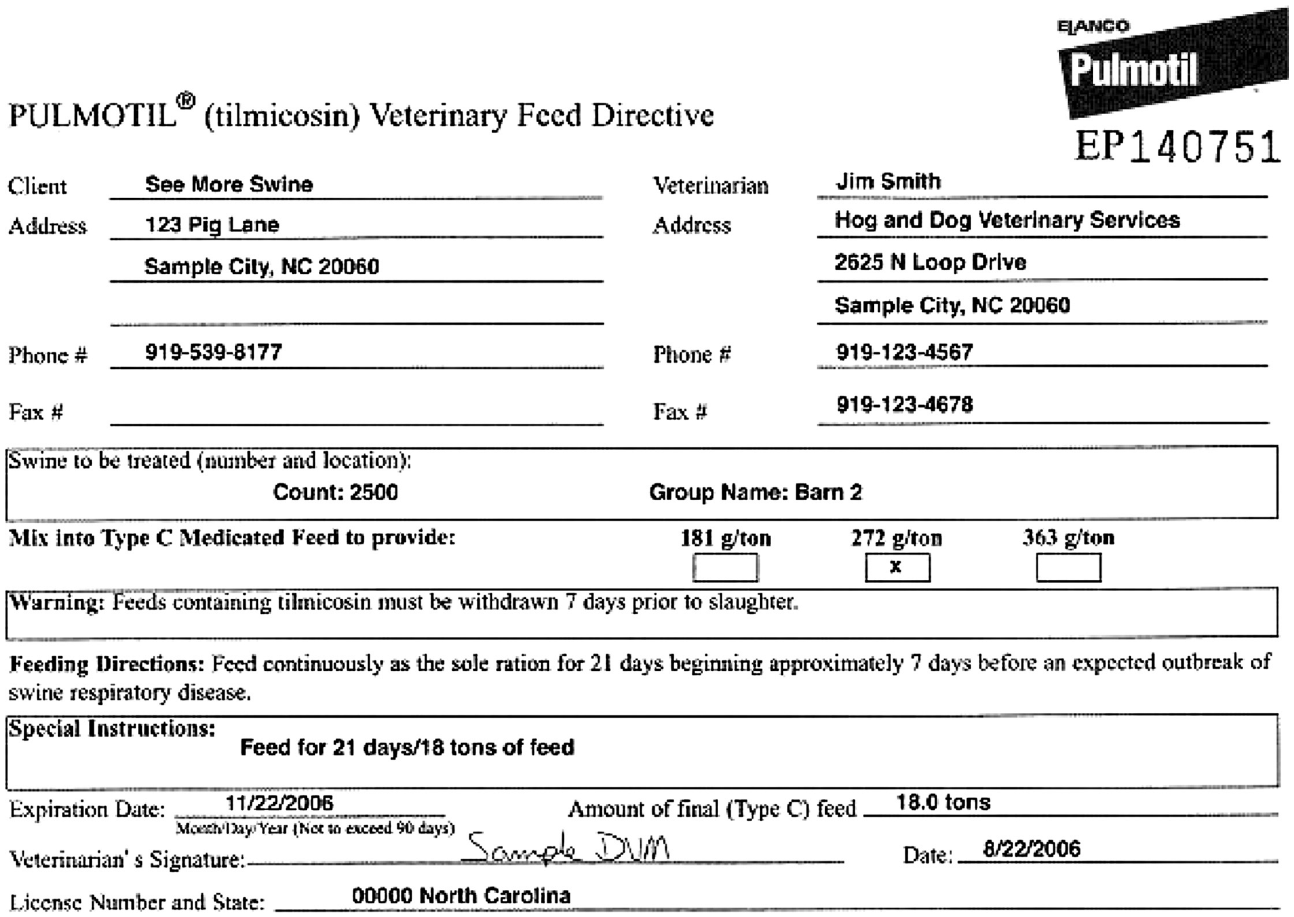
1) The person or firm supplying a VFD drug to a producer must receive and retain a copy of the signed VFD form issued by the veterinarian.
2) Licensed feed manufacturers and distributors who ship VFD feed to a downstream distributor or retailer for inventory must receive and retain a copy of written acknowledgement stating that the VFD feed will be further distributed only in accordance with FDA requirements.
3) All distributors and retailers who do not hold a feed mill license must notify the FDA within 30 days of their initial VFD feed shipment.

The form for Veterinary Feed Directive products should be provided to the veterinarian by the manufacturer of the specific therapeutic agent (Figure 59.2). This form must be completed by the veterinarian and should normally include the following information:

1) the name, address, and telephone number of the producer;
2) the species, location, number of, and description of animals being treated;
3) the date(s) of treatment (if different from the date on the VFD form);
4) the name of the drug to be used, the concentration of the drug to be included in the feed, and the amount of feed to be manufactured;
5) the directions for mixing and feeding (including dilution) and duration of treatment, including appropriate withdrawal times; the expiration date of the feed;
6) the veterinarian’s name, address, license number, state of licensing, and signature.

The form is the most significant component of using VFD approved medications. A VFD order cannot be issued by telephone. If the form cannot be hand-delivered, it can be faxed or sent via an email attachment; however, the original form must be received by the distributor within 5 working days. VFD forms have to be retained for a minimum of 2 years by all three parties involved (the veterinarian, the producer, and the feed mill) and should be available for review by the FDA.

Only vendors with a valid feed mill license are authorized to store VFD products, which means that a producer is not allowed to keep a surplus of medicated feed



ELANCO Pulmotil

EP140751

PULMOTIL® (tilmicosin) Veterinary Feed Directive

Client	See More Swine	Veterinarian	Jim Smith
Address	123 Pig Lane	Address	Hog and Dog Veterinary Services
	Sample City, NC 20060		2625 N Loop Drive
			Sample City, NC 20060
Phone #	919-539-8177	Phone #	919-123-4567
Fax #		Fax #	919-123-4678

Swine to be treated (number and location):
Count: 2500 Group Name: Barn 2

Mix into Type C Medicated Feed to provide: **181 g/ton** ☐ **272 g/ton** ☒ **363 g/ton** ☐

Warning: Feeds containing tilmicosin must be withdrawn 7 days prior to slaughter.

Feeding Directions: Feed continuously as the sole ration for 21 days beginning approximately 7 days before an expected outbreak of swine respiratory disease.

Special Instructions: Feed for 21 days/18 tons of feed

Expiration Date: 11/22/2006
Month/Day/Year (Not to exceed 90 days)
Amount of final (Type C) feed 18.0 tons
Veterinarian's Signature: Sample DVM
Date: 8/22/2006
License Number and State: 00000 North Carolina

Figure 59.2 Sample Veterinary Feed Directive form for tilmicosin. Source: © Copyright Eli Lilly and Company. All Rights Reserved. Used with Permission.

on the farm. However, licensed veterinarians are allowed to act as distributors of VFD feeds by notifying the FDA once, in writing, 30 days before the first anticipated distribution of medicated feed. More information on VFD regulation can be find in the FDA Guidance for Industry 120 document available at the FDA website: www.fda.gov/downloads/AnimalVeterinary/GuidanceComplianceEnforcement/GuidanceforIndustry/UCM052660.pdf.

As VFDs become more common the above process may change. Plans and resources for electronically generated VFD forms are currently underway but have not been fully developed. Companies such as GlboalVetLink are working to develop programs by which computer generated VFD forms could by written by veterinarians and distributed to both the client and feedmill with copies automatically saved on a database for an extended period of time. Certainly, the coming years will see a significant expansion in the use of VFD drug across all major food animal species.

Site-Directed Therapy

The final consideration related to veterinary dosage forms are those drugs specifically designed for local effects. These include intramammary and intrauterine formulations, and ophthalmic and otic drugs. Examples of these are discussed under the individual drug sections and special therapy chapters of this text.

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60

Evidence-Based Pharmacotherapy

Ronette Gehring

Introduction

Evidence-based veterinary medicine (EBVM) is a formal approach to integrating the best research evidence with clinical expertise and client perspectives to provide high-quality veterinary services. This chapter will focus on the application of EBVM principles to pharmacotherapy (the selection of pharmacological treatments and dosage regimens). The EBVM approach guides the busy practitioner through the massive amount of constantly and rapidly evolving scientific data, so that he/she can make the best clinical decisions possible by focusing on information that is directly related to specific patient problems (Cockcroft and Holmes, 2003).

How is EBVM Different from Other Approaches to Pharmacotherapy?

It seems obvious that treatment choices should be based on the best available evidence, but many approaches to clinical practice can result in biased or incomplete assessment of available options. These approaches include, but are not limited to, relying on standardized protocols, perusing the information about drugs that is provided by pharmaceutical companies, basing decisions on pathophysiological rationale or unsystematic clinical observations of previous cases, seeking advice from colleagues and experts, and performing unstructured appraisals of information in textbooks, journals, and online resources. Personal experience is subjective and can be misleading, predictions based on pathophysiological rationale need to be validated with randomized controlled clinical trials, and evidence from the scientific literature needs to be systematically evaluated to identify poorly designed studies, poor-quality data, or biased analyses.

EBVM sets itself apart from other approaches to clinical practice in that it emphasizes the systematic review of all available evidence. Randomized controlled clinical trials reported in the scientific literature are the preferred evidence, but, if these do not exist, evidence from expert opinion, case reports, personal experience, and other nonliterature-based sources can be considered. Regardless of the source, all evidence needs to be collated, assessed, and ranked to support objective decisions regarding the treatment of patients.

How is EBVM Applied to Selecting the Best Treatment for a Specific Patient?

EBVM can be defined as “the conscientious, explicit, and judicious use of current best evidence in making decisions about the care of individual patients” (Sackett et al., 2000). This means that clinicians should follow an explicit and methodical approach to their clinical decision making so that they can explain how and why one treatment option was chosen over another. There are three foundational skills that are required to do this:

1) Be able to identify information needs and transform them into questions.
2) Know where to seek the evidence needed to answer these questions.
3) Be able to understand and critically appraise (judge the quality) all the evidence.

Transforming Information Needs into Questions

One of the key skills for applying the EBVM approach to clinical practice is translating information needs that arise from a clinical scenario into questions that can guide the search for evidence. The following are some examples of clinical questions that are focused on pharmacotherapy:

1) Is drug X effective for treating condition Y?
2) Is the efficacy of drug X worth the harm (adverse effects) it causes in treating condition Y?
3) Is the efficacy of drug X worth the cost of treatment for condition Y? (i.e., Is drug X an efficient way to treat condition Y?)

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4) How exactly should drug X be used in treating condition Y?
5) What is the place of drug X in the therapy for condition Y?
6) Can drug X cause problem Y?
7) What other drugs may be helpful in a patient who has not responded to drugs X, Y, and Z for condition B?
8) What is the expected outcome of drug X?

Once the question has been formulated, appropriate search terms can be identified to ensure that a search of the scientific literature and other information sources is productive and thorough, producing relevant results.

Finding Evidence to Answer the Questions

Traditional sources of information for practicing veterinarians include student and course notes, print journals, and textbooks. These may all be valid sources, depending on the clinician's needs, but the disadvantage is that they can quickly become outdated and/or are costly to access and update. More recently, the Internet and electronic media have become relatively inexpensive and convenient sources of scientific information, making it possible to access the scientific literature without going to a physical veterinary library. In addition, information on the Internet or an electronic resource is easier to update compared to its printed counterpart, and is easy to search. This can, however, produce a glut of information about any particular subject, requiring a lot of effort to sift through and discern its relevance, quality, and validity. Despite this disadvantage, a survey conducted by the American Veterinary Medical Association in 2008 found that 88% of veterinarians thought that the Internet enhanced their access to the latest scientific information and research (AVMA, 2008).

A survey conducted in 1996 found that medical doctors often had the need for more information about treatments and drugs during consultations, but searching for information was time consuming. Also, the available information could be overwhelming and difficult to evaluate (Smith, 1996). Veterinarians probably face similar challenges. Ideal information sources would therefore be directly relevant to the veterinarian's practice, valid, and require minimal time and effort to access (Shaughnessy et al., 1994). Based on this assumption, highly useful types of information would be regularly updated, evidence-based electronic textbooks, systematic journal reviews, portable summaries of systematic reviews, and online Internet reviews. Print media seems less useful as it is more difficult access, and primary sources (e.g., journal articles) require time and effort to collate and critically appraise for validity.

Since the conduct of these surveys, more high-quality online veterinary medical information resources have become available. Links to these resources can be found on the website of the Evidence-Based Veterinary Medical Association (EBVMA), a community dedicated to evidence-based veterinary medicine based in the United States (https://ebvma.org). Resources focused on veterinary drugs remain scarce, but there are growing databases of reviews of current best evidence to answer specific clinical questions (e.g., BestBETs for Vets, a project run by the Centre for Evidence-based Veterinary Medicine at the University of Nottingham, available at: http://bestbetsforvets.org). Evidence summaries of the literature pertaining to contentious label and extralabel use of drugs for species-specific indications are also available as part of the drug information monographs developed by the United States Pharmacopeia (USP). These monographs are no longer updated by the USP, but the historic documents are archived on the website of the American Academy of Veterinary Pharmacology and Therapeutics (AAVPT) (www.aavpt.org). The evidence summaries have been digitized into an online database to facilitate searching and updating and a link is also available at www.aavpt.org.

Appraising the Evidence

Once all the available information related to a clinical question has been gathered, it needs to be critically appraised for quality and validity, and weighted according to the statistical and methodological rigor of the studies that generated the data. Ideally, only data from the scientific literature should be considered, but this may not always be possible in veterinary medicine due to the scarcity of relevant clinical studies. Expert opinion, case reports, personal experience and other nonliterature-based sources can therefore also be considered, but need to be evaluated, ranked and weighted accordingly in the clinical decision-making process.

The classical evidence hierarchy is shown in Figure 60.1. In this classification, the systematic review is considered the pinnacle of evidence as it combines the results of different studies in a rigorous and systematic manner to best understand how the results of clinical studies represent reality. These are rare in veterinary medicine, particularly with regards to understanding the value of drugs for the treatment of specific conditions in different species.

A notable exception to the scarcity of EBVM resources in veterinary pharmacotherapeutics is the collection of Veterinary Drug Information Monographs originally published by the United States Pharmacopeia. The goal of developing these monographs was to create a comprehensive single source of veterinary information about a drug. The process incorporated evidence-based approaches with interpretation of the evidence by an

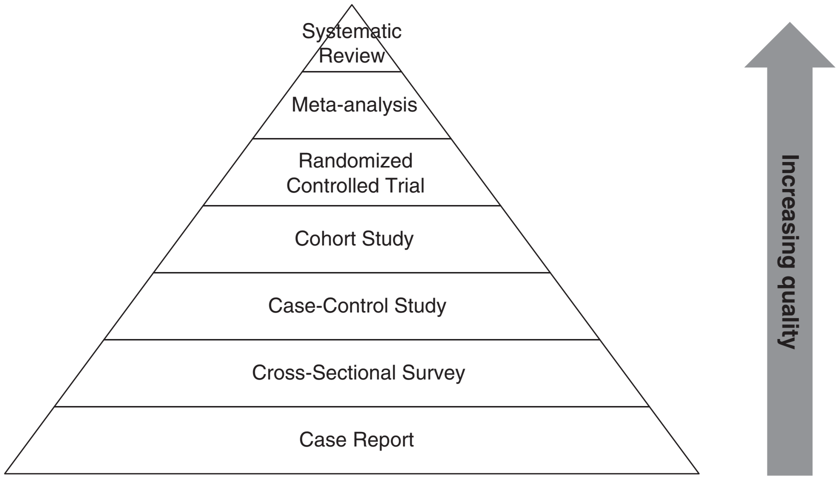

Figure 60.1 The evidence hierarchy.

Table 60.1 Evidence ratings

Evidence quality
A. Good evidence to support a recommendation for use
B. Moderate evidence to support a recommendation for use
C. Insufficient evidence to support a recommendation for use
D. Moderate evidence to support a recommendation against use
E. Good evidence to support a recommendation against use

Evidence type
1. Species-specific evidence from at least one large randomized and controlled trial (RCT) or multiple small RCTs
2. Species-specific evidence from a small RCT, disease models, large case studies, or pharmacokinetic studies using surrogate endpoints or evidence from well-designed trials in a different species that is considered appropriate for comparison
3. Dramatic results from either well-designed, species-specific trials without controls or small case studies
4. Pharmacokinetic studies without surrogate endpoints
5. In vitro studies
6. Opinions of respect authorities on the basis of clinical experience or reports of expert committees

expert committee (earlier editions of this textbook contain detailed descriptions of the process).

The indications section of these monographs was where the EBVM approach was most strongly applied. For all extralabel indications, and for any label indication that was viewed as contentious by a member of the expert committee, tables summarizing the evidence in support of that indication from the scientific literature were generated. Each table contained the citation information, the type of study (e.g., case report, clinical trial, disease model, in vitro study, metaanalysis, pharmacokinetic study with surrogate endpoints, pharmacokinetic study without surrogate endpoints), whether treatments were randomized, type of control used (e.g., positive, negative, uncontrolled), and the type of masking used (e.g., single-masked, double-masked, nonmasked). A synopsis of the methods used, doses and duration of therapy studied, results, author conclusions, and limitations noted by the USP staff were included. Indications were then classified as “Accepted”, “Potentially effective” or “Unaccepted”, and assigned an evidence rating based on the quantity and quality of evidence. The evidence rating consisted of a letter (A–E) and a number (1–6) as indicators of the evidence quality and type, respectively. The evidence rating system is summarized in Table 60.1.

Funds are no longer available for the development of new veterinary drug information monographs, nor are the existing ones being updated anymore. But they remain a unique resource, and offer a framework for the rigorous evaluation of available information for veterinary drugs. It is hoped that, in the future, resources will be found to continue this important work.

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61

Chemical Residues In Tissues Of Food Animals

Jim E. Riviere

Most of this textbook has focused on describing the pharmacodynamics and pharmacokinetics of drugs in animals. In both human medical and companion-animal veterinary practice, the primary concern in drug selection and use is the therapeutic end point, whether or not the drug is efficacious against the disease being treated. Doses are usually administered at label recommendations, and, if greater than label doses are administered, only potential toxicity is of concern. While this line of reasoning is also true to a large degree in food-animal production, veterinarians and producers involved in the treatment of disease in food animals bear the additional concern of the persistence of drug residues in the edible tissues after the disease process has been treated. Adulteration of the food supply with antimicrobial agents, pesticides, environmental contaminants, and other chemicals has been a growing source of concern to the general public and special-interest groups in recent years.

The importance of chemical residues in the edible tissues of food-producing animals has been thoroughly reviewed elsewhere (Bevill, 1989; Sundlof, 1989; Van Dresser and Wilcke, 1989; Mercer, 1990; Riviere, 1991, 1992a; Kindred and Hubbert, 1993; Baynes et al., 2015) and has been the topic of a recent text by the author (Baynes and Riviere, 2014). The purpose of this chapter is to acquaint the veterinarian with the legal and regulatory issues concerning the control of drug and other chemical residues in the United States, and to review some of the pharmacokinetic parameters used to determine withdrawal times for drugs and other chemicals in food animals. Drug use in food animal practice is fully discussed in Chapter 52 and the legal regulations are also extensively covered in Chapter 55 of this text. All of these references should be consulted for an in depth analysis of this complex and ever changing topic.

The primary parameter used by veterinarians to prevent violative tissue residues is the length of the withdrawal time, or the time required for a drug after dosing to be depleted from the animal before the animal's meat can be marketed for human consumption. In dairy practice, this is the milk discard time. Recently, government regulation and control as well as adoption of more sensitive multiresidue analytical tools have necessitated more stringent adherence to withdrawal times, and on-site monitoring for many drugs has been instituted. This may have an economic impact on production costs. Unlike in previous editions of this text, withdrawal times will not be tabulated, because they are subject to constant regulatory revision. Extensive tables of tissue depletion pharmacokinetic data are published elsewhere (Craigmill et al., 2006). Current withdrawal times are provided on the Food Animal Residue Avoidance Databank (FARAD) website, available at: www.farad.org.

The Concern Over Residues in Food

A great deal of concern has been demonstrated over the last 50 years about the presence of chemical adulterants or residues, mainly antimicrobials and pesticides, in the meat, poultry, and milk supplies of the United States. By definition, a chemical residue is either the parent compound or metabolite of that parent compound that may accumulate, deposit, or otherwise be stored within the cells, tissues, organs, or edible products (e.g., milk, eggs) of an animal following its use to prevent, control, or treat animal disease, or to enhance production. Residues can also result from unintentional administration of drugs, or food additives. Finally, accidental exposure to chemicals in the environment can also result in tissue residues.

Concerns over food residues are economic as well as public health related. For example, the contamination of milk with antibiotics, most commonly penicillin, can affect starter cultures used to make fermented milk products such as cheeses, buttermilk, sour cream, etc., which can result in economic losses to those processors. From a public-health viewpoint, both the US government and producer associations have taken active roles in minimizing antibiotic residues in meats and milk. Penicillin, for example, is known to induce allergic reactions in some sensitive people, and therefore,

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Companion Website: www.wiley.com/go/riviere/pharmacology

penicillin-tainted milk poses a health risk for these individuals. Similarly, chloramphenicol has been reported to induce blood dyscrasias that may lead to death; hence, its use in food-producing animals has been prohibited by the Food and Drug Administration (FDA). The FDA has also prohibited the use of nitrofurans in food-producing animals (excluding the topical routes for administration) because studies have shown them to be carcinogenic. Not only therapeutic drugs but pesticides create residue problems. Most pesticides are administered topically, allowing some amount of percutaneous absorption and sequestration in edible tissues. Lindane has been detected in the fat deposits of sheep dipped in a 0.0125% lindane emulsion 12 weeks after topical exposure (Collett and Harrison, 1963). Other studies have shown lindane residues in sheep, goats (Jackson et al., 1959), and lactating cows (Oehler et al., 1969). In addition to lindane, many common pesticides (organochlorines, organophosphates, botanicals, pyrethrins, etc.) and herbicides used in agriculture today that are applied topically have been shown to produce residues in food-producing animals. Environmental contaminants (e.g., heavy metals, PCB, dioxins, mycotoxins) are also of major concern today. Tissue residue violations detected by governmental monitoring programs have been summarized by Sundlof (1989) as well as Baynes et al. (2015).

The USDA Food Safety and Inspection Service (FSIS) manages the National Residue Program (NRP) and yearly publishes sampling plans for the next year (Blue Book) and sampling results for the prior year (Red Book). Both public-health and economic concerns have been the major driving forces in the United States and in other countries behind the search for ways to minimize the threat of residue contamination of the public food supply. The latest editions of these reports (2014, 2015) have served as the source of some NRP details in the present chapter.

Contamination of the food supply with chemical residues is rarely an intentional act and usually results either from failure to observe the correct meat withdrawal or milk discard time for a drug after it has been used to treat a disease process in food animals or from accidental contamination of feed by chemicals or drugs. An early study by Van Dresser and Wilcke (1989) provides some interesting insight on drug residue problems in food-producing animals. In that study, streptomycin, penicillin, sulfamethazine, and oxytetracycline were the four most common antibiotics found in tissues, with sulfamethazine being the most commonly found sulfonamide in animal tissues. Long-acting formulations of these drugs (i.e., penicillin and oxytetracycline) had the highest association with violative residues in the animals involved in the study. Injectable drugs were more likely to be associated with residue problems than were feed additives and boluses. Most of these residues were found in veal calves, cows, and market barrows and gilts. The most frequently cited reason for violative residues was failure to observe the correct withdrawal time for the drug. Failure to observe the correct withdrawal time was cited as the most common reason for violative drug residue levels in a study performed by the FDA in the 1970s (Bevill, 1984) and continued to be the most common cause of residue violations in the 1990s. Interestingly, in this study the producer was found to be the responsible party in 80% of the cases investigated for violative levels of drug found in edible tissues (when the responsible party could be identified); unapproved drug use (extralabel drugs) is not considered a major cause of drug residues in animals. Ways to prevent drug residues will be discussed in Section Residue Prevention.

The FDA and Environmental Protection Agency (EPA) establish tolerances for a drug, pesticide, or other chemical in the relevant tissues of the food-producing animals. The tolerance is the tissue concentration below which a marker residue for the drug or chemical must fall in the target tissue before that animal's edible tissues(s) (meat, milk, or eggs) are considered safe for human consumption (Riviere, 1991). The marker residue may be the parent compound, or a metabolite, and reflects a known relationship to the total residues of the drug or chemical (parent and all metabolites), although this has recently been suggested to not be constant but instead sensitive to disease and other pathophysiological conditions seen in target animals (Lin et al., 2016). The target tissue is an edible tissue, frequently liver or kidney which, when the compound has depleted below the tolerance, assures that all edible tissues are safe for human consumption. Tolerances for different tissues are considered legal end points for which drug withdrawal times are established. Tolerances are established based on assessment of potential hazard of consumption to humans, a topic extensively reviewed elsewhere (Gehring et al., 2006; Baynes and Riviere, 2014). Oral toxicity studies are conducted in animals leading to the determination of an acceptable daily intake (ADI) for the compound in the human diet. These studies consider the compound's carcinogenic potential, systemic, reproductive, and developmental toxicity, and incorporate various safety factors. Recently, the potential for an antimicrobial compound to induce resistance in bacteria is also factored in. A safe concentration for human consumption is calculated using an equation that accounts for the amount of a specific food consumed by a person representing a high-consuming population (e.g., 19-year-old males) so that a safe concentration of the drug in this food (e.g., meat, milk, eggs) can be established. Various safety factors and statistical considerations are built into these determinations, and a tolerance for this drug in the specific tissue is established where appropriate and published. For example, safety factors reflect the duration of exposure and the nature of the toxic effects associated with the chemical. A teratogen requires a larger safety factor than a nonteratogen, and ADIs based on a short-term subchronic study use a larger safety factor than ADIs based on chronic studies. The tolerance is for a specific drug or chemical entity and reflects both the inherent toxicity of the chemical as well as assumptions about the human consumption of the tissue for which the tolerance is established. Tolerances are determined for the active ingredient (the drug substance or its metabolite) and are not established for the specific formulation of the commercial drug product. A full discussion of this process is presented in the latest FDA Guidance *General Principles for Evaluating the Human Food Safety of New Animal Drugs Used in Food-Producing Animals – July, 2016;* it has also been reviewed (Baynes et al., 1999; Baynes and Riviere, 2014) and can be found in toxicology or risk assessment texts. It is important for the veterinarian to realize that the end point for determining withdrawal times, the tolerance, is a combined scientific and legal concept and therefore is ultimately controlled by regulatory and not purely scientific and medical practices.

The actual withdrawal time appearing on a drug label is also a function of the experimental design that the manufacturer used in the research studies submitted to the FDA for approval. Thus, although the science governing the withdrawal time is based on the pharmacokinetic principles discussed below, the withdrawal time is actually determined based on experimental data. Generally, a drug is administered to healthy animals, groups of the animals are slaughtered at sequential time intervals, and their edible tissues are analyzed for drug concentrations. The withdrawal time is the time from cessation of treatment to the time it takes for the residues of the drug to deplete below the safe concentration. A statistical method (*statistical tolerance limit procedure*) is used to determine the time at which the marker residue depletes to the tolerance in the target tissue. The method determines the time, rounded up to the next whole day, at which the upper bound of the marker residue tissue concentration is below the established tolerance in the target tissue (where the upper bound is statistically determined to represent with 95% confidence the 99th percentile of the population). Withdrawal times for the FDA-approved drugs for use in food animals are only valid for the specified species, dose, route, and frequency of administration. They are also specific to the manufacturer's product and formulation; thus, a drug substance (the active ingredient) may have different withdrawal times when present in differently formulated drug products. An analogous process occurs in establishing milk discard times and in determining the withdrawal times for drugs administered to egg-laying poultry (although presently, all drugs approved in the USA for use in laying hens have a 0-day withdrawal).

Regulation of Drug Residues in Animals

Producers, veterinarians, and other persons involved with chemicals and food-animal production should be acquainted with a few terms and the agencies involved in drug residue control in order to better understand the drug residue problem and how withdrawal times are determined.

The use of drugs in veterinary medicine, especially in food-producing animals, is closely regulated in the United States by the FDA under the Department of Health and Human Services. The FDA is charged, through the Federal Food, Drug and Cosmetic Act, with regulating the use of drugs in humans and in animals as well as requiring that the safety and efficacy of a drug be established before the product can be approved for use in animals, including those drugs added to animal feeds. The FDA also regulates human biologics, medical devices, drugs, and food safety. The FDA's Center for Veterinary Medicine (FDA-CVM) is responsible for the regulation of drugs, medical devices, and feeds intended for animals. The FDA is charged with the responsibility for establishing withdrawal times for drugs and the tolerances of drugs. The FDA and the EPA share responsibility for establishing tolerances for pesticide residues in animal-derived foods.

Whereas the FDA establishes safety guidelines for drug use in food animals, it is the responsibility of the USDA to enforce the standards established by the FDA and the EPA. The USDA, through authorization by the Federal Meat Inspection Act and the Poultry Inspection Act, inspects meat and poultry for sale through interstate commerce. The USDA is also authorized to test the tissues of food-producing animals through provisions in the Federal Insecticide, Fungicide and Rodenticide Act to determine whether violative levels of residues of chemicals and drugs are present. The USDA FSIS monitors these tissues through the NRP and identifies problems with drug and chemical residues. The NRP has been in existence for over 30 years and has concentrated on individual as well as population sampling for monitoring and surveying possible residue problems in slaughter animals. In 1992, the NRP estimated they would collect some 350,000 specimens that year to analyze for antimicrobial and pesticide residues (Kindred and Hubbert, 1993). The FSIS is the largest safety inspection force in the federal government (Norcross and Post, 1990). A few years ago, FSIS introduced a new three-tiered monitoring plan and more sensitive multiresidue analytical methods. Sampling occurs in a random commodity-specific national plan, as well as at the production and herd level. The number of samples and selection criteria have changed in 2014–2015 and thus comparisons to previous data may be problematic.

There are a number of rapid screening test, once used by FSIS and in producer food safety programs. The FSIS has used several rapid tests for determining contamination of animal products. Among these are the Swab Test on Premises (STOP) for antibiotic and sulfonamide residues, the Overnight Rapid Beef Identification Test (ORBIT) for species identification of meat, the Calf Antibiotic and Sulfa Test (CAST), the Sulfa-on-Site (SOS), and a variety of enzyme-linked immunosorbant assays (ELISAs) (Norcross and Post, 1990). Milk in bulk tanks and from individual animals is assayed for antibiotic residues using various testing strategies: Penzyme III (SmithKline Beecham Animal Health), Spot Test (Cambridge Biotech Corp.), Charm Tests (Charm Sciences, Inc.), and Delvo tests (Gist-Brocades Food Ingredients, Inc.), to name only a few. This area is difficult to adequately summarize because rapid advances in analytical screening methodologies have led to the rapid development of new tests.

Other federal government agencies have defined roles in regulating the sale and use of drugs in animals, including the Drug Enforcement Agency (a division of the Department of Justice), the Animal and Plant Health Inspection Service (a part of the USDA), the EPA (mainly for pesticides), and the Department of Transportation. On a state level, the Department of Public Safety, Department of Health, the Animal Health Commission, Boards of Veterinary Medical Licensing, and Pharmacy Boards all have some regulatory influence over the use of drugs in food-producing as well as companion animals. Other legal jurisdictions, including the European Union, have established protocols that should be consulted for details in these countries. As in the USA, these regulations are fluid, end points change based on residue sampling results and new drug submissions. One should always consult the relevant government websites for the latest information.

The Veterinarian and Extralabel Drug Use

In the USA, the FDA approves new animal drugs for specific indications in a particular species or subclass of animals (e.g., dairy cattle, weanling pigs, etc.). Occasionally, veterinarians may encounter diseases or conditions in animals for which there are no FDA-approved drugs. Under such circumstances, US veterinarians often administer drugs that are not approved for use in food animals or they administer approved drugs in nonapproved ways, practices commonly referred to as “extralabel” usage. Extralabel drug use is defined as the use of a drug in a manner that is inconsistent with its FDA-approved labeling, a practice that, until 1994, was technically illegal. Recognizing that the Food, Drug and Cosmetic Act (FD&C) placed veterinarians in an untenable position of having to choose between providing for relief of animal suffering or complying with the law, the US Congress passed the Animal Medicinal Drug Use Clarification Act (AMDUCA) in 1994. AMDUCA amended the FD&C Act to allow veterinarians under specific conditions to prescribe and administer drugs in an extralabel manner. Table 61.1 lists drugs specifically prohibited from extralabel use in food animals. Under AMDUCA, veterinarians may resort to the extralabel use of approved animal and human pharmaceuticals provided that the following conditions are met:

1) There is no approved new animal drug that is labeled for the intended use and that contains the same active ingredient which is in the required dosage form and concentration, except where a veterinarian finds, within the context of a valid veterinarian–client–patient relationship, that the approved new animal drug is clinically ineffective for its intended use.
2) Prior to prescribing or dispensing an approved new animal or human drug for an extralabel use in food animals, the veterinarian must:
   - make a careful diagnosis and evaluation of the conditions for which the drug is to be used;
   - establish a substantially extended withdrawal period prior to marketing of milk, meat, eggs, or other edible products supported by appropriate scientific information, if applicable;
   - institute procedures to assure that the identity of the treated animal or animals is carefully maintained; and
   - take appropriate measures to assure that assigned timeframes for withdrawal are met and no illegal drug residues occur in any food-producing animal subjected to extralabel treatment.

The following additional conditions must be met for a permitted extralabel use in food-producing animals of an approved *human* drug, or of an animal drug approved only for use in animals not intended for human consumption:

1) Such use must be accomplished in accordance with an appropriate medical rationale.
2) If scientific information on the human food safety aspect of the use of the drug in food-producing animals is not available, the veterinarian must take appropriate measures to assure that the animal and its food products will not enter the human food supply.
3) Extralabel use of an approved human drug in a food-producing animal is not permitted if an animal drug approved for use in food-producing animals can be used in an extralabel manner for the particular use.

Implicit in these regulations are that the veterinarian makes every effort to first use an approved drug at an approved dosage, that an individual veterinarian–client–patient relationship exists between the producer

Table 61.1 Drugs prohibited from extralabel use (ELDU) in food animals. Source: Adapted from Food Animal Residue Avoidance Databank, 2016.

Drugs with no allowable extralabel uses in any food-producing animal species

- **Chloramphenicol**
- **Clenbuterol**
- **Diethylstilbesterol (Des)**
- **Fluoroquinolone-class antibiotics**
- **Glycopeptides**
  - all agents, including **Vancomycin**
- **Medicated feeds**
- **Nitroimidazoles**
  - all agents, including **dimetridazole**, **ipronidazole**, **metronidazole**
- **Nitrofurans**
  - all agents, including **furazolidine**, **nitrofurazone,** and others

Drugs with restricted extralabel uses in food-producing animal species

- **adamantane and neuraminidase inhibitors** in all poultry, including ducks
  - these agents are approved for treatment or prevention of influenza A
- **Cephalosporin-class antibiotics** ***except*** **cephapirin** in all classes of cattle, chickens, pigs, and turkeys
  - ELDU restrictions apply to all production classes of major food-animal species
    - no ELDU for purpose of disease prevention
    - no ELDU that involves unapproved dose, treatment duration, frequency, or administration route
    - agent must be approved for that species and production class
  - ELDU restrictions *do not apply* to minor-use food animal species
- **Gentian violet** – prohibited from use in food or feed of food-producing animal species
- **Indexed drugs** – some exceptions for minor-use species
- **Phenylbutazone** – in female dairy cattle (20 months of age or older)
- **Sulfonamide-class antibiotics** – in lactating dairy cattle – approved uses are allowed for sulfadimethoxine, sulfabromomethazine, and sulfaethoxypyridazine

and the veterinarian, that proper animal identification and documentation be maintained, and that the veterinarian makes every effort to determine an extended withdrawal time for this usage (e.g., consult with a resource such as the Food Animal Residue Avoidance Databank, FARAD), which the producer must adhere to. The regulation prohibits extralabel use for routine production purposes, routine disease prevention, or as feed additives.

Pharmacokinetics and Residues

Pharmacokinetics is the science of quantitating the change in drug concentration in the body over time as a function of the administered dose. Although this discipline has been covered in Chapters 2 and 3 of this text and elsewhere (Riviere, 1992b, 1999, 2011; Baynes and Riviere, 2014), it is important to review some principles here since it serves as the basis for understanding withdrawal times.

How a drug or combination of drugs behaves in the body after administration not only is important from a therapeutic point of view but is of paramount importance to the producer and veterinarian in order to prevent residues in the edible tissues after the disease process has been resolved and the animal is slaughtered. For therapeutic usefulness and drug residue determinations, a known amount of drug is administered to a healthy animal. Serum concentration data are collected and mathematical models are created so that the overall disposition of the drug in the body can be evaluated in relation to absorption, distribution, metabolism, and elimination. Parameters for these models are estimated by fitting regression lines to the observed serum concentration versus time profiles, an example of which is shown in Chapter 3, Figure 3.15.

The slopes of the three lines shown in Figure 3.15, when plotted on a semilogarithmic plot, represent distribution (λ_1), short-term elimination (λ_2), and long-term elimination (λ_3) from the body. The λ_1 (or α) and λ_2 (or β) phases of the serum concentration versus time profile are the only phases that are usually present when serum concentrations are monitored over a short period of time after dosing and are typically used to predict therapeutic drug concentrations. When serum concentrations are monitored for longer periods of time after drug administration using more sensitive analytical assay methods, however, the λ_3 (or γ) phase of elimination appears for certain compounds. This can be present for several days or several months after the last administered dose depending on the drug's physicochemical properties, the amount of drug administered, and the species in which it was administered. This λ_3 phase reflects drug disposition in the so-called "deep compartments." Alternatively, for prolonged-action depot preparations (e.g., benzathine penicillin), this terminal phase may actually reflect the rate-limiting absorption phase ("flip–flop" phase). For purposes of determining withdrawal times of drugs that may be used in food-producing animals, we will focus our discussion on the terminal phase of drug elimination to determine the half-life ($t_{1/2}$) of the drug in the body, since this is the measurement that is relevant in determining drug withdrawal times.

In the actual research studies conducted to determine a withdrawal time, it is the half-life of drug in the specific tissue that is of paramount importance. This information is not available to the veterinarian. Additionally, tissue pharmacokinetics are exceedingly complex, and a discussion here of terminal half-lives will be sufficient to illustrate the concepts involved. For more in-depth information on the clinical application of pharmacokinetics and how pharmacokinetic parameters can affect clinical parameters, readers should consult Chapters 2 and 3 of this textbook.

The half-life of the drug or chemical in the body is the primary biological measurement used to determine withdrawal times of drugs and chemicals in food-producing animals; however, this parameter can be influenced by many biological factors (Baynes and Riviere, 2014; Baynes et al., 2015). The $t_{1/2}$ of a drug by definition is the time is takes for 50% of the drug in the animal to be eliminated from the body and is based on the terminal slope of the elimination curve used to determine the withdrawal time of a drug. The $t_{1/2}$ is calculated using the equation

$$t_{1/2} = \ln 2/\text{slope or } t_{1/2} = 0.693/\text{slope}$$

If the concentration of a drug in the muscle of a food animal after dosing is 100 parts per million (ppm), then the time it takes for the concentration in the muscle to decrease to 50 ppm would be the biological half-life of the drug in the muscle. Extrapolated out, the amount of drug in the muscle after 10 half-lives would be 0.1 ppm, or, put in other terms, 99.9% of the drug would have been eliminated from the muscle after 10 half-lives. If the dose is doubled and the beginning concentration in the muscle is now 200 ppm, only 1 additional half-life would be required to reach the 0.1 ppm concentration. On the other hand, if the half-life of the drug in the muscle is doubled, perhaps due to a disease state, then the elimination half-life would also double, thereby increasing the risk of violative drug residues in the edible tissues of that animal. These two scenarios, if properly understood, are simple tools to reduce violative residues when drugs are used in an extralabel fashion or according to label in severely diseased animals.

As stated above, the elimination half-life can be influenced by many biological factors. The half-life of a drug or chemical in the body is influenced by how well it distributes in the body and how quickly it is eliminated from the body. The physicochemical properties of a drug can influence its disposition in the body – in particular, how well it distributes into certain tissues, whether or not it penetrates intracellularly, or whether it permeates the blood–brain barrier. The volume of distribution (V_d) is the quantitative estimate of the extent of the distribution of the drug in the body and can therefore directly influence the $t_{1/2}$ of the drug. It is a proportionality constant relating the concentration of drug in the serum to the total amount of drug in the body. For an intravenous injection of a drug, the equation for calculating V_d is

$$V_d = \text{amount of drug in the body/}\\ \text{serum drug concentration}$$

It is important to point out that the V_d, typically reported in l/kg, does not actually refer to any specific physiological space or body area; rather, it gives a good indication of how well a drug in general distributes throughout the body. A drug that has a large V_d typically has good tissue distribution throughout the body (e.g., tetracyclines), while a drug with a small V_d has less penetration into the body tissues as whole, perhaps being confined to the extracellular spaces due to one or more of its physicochemical properties (not lipid soluble, fixed charge). While V_d may give an indication of the overall distribution of a drug, some drugs may not be uniformly distributed throughout the body. In this case, a drug may seek specific cells or organs or be bound to tissue macromolecules, resulting in a large V_d measurement and yet a relatively poor overall distribution in a majority of the body's tissues. Some drugs may have prolonged withdrawal times due to a large V_d.

In addition to V_d, the clearance (Cl) of the drug also plays an important part in determining the withdrawal time of the drug. Clearance quantitates the efficiency of the elimination processes and is defined as the rate of drug elimination from the body relative to the concentration of drug in the serum by the equation

$$Cl = \text{rate of elimination/serum drug concentration}$$

Drugs that have a slow rate of elimination from the body will tend to have protracted half-lives, while those that are eliminated quickly will have shorter half-lives.

The $t_{1/2}$ is dependent on two functions: V_d and Cl. By combining terms, an equation can be derived that reflects the influence of V_d and Cl on the $t_{1/2}$ of a drug:

$$t_{1/2} = \ln 2V_d/Cl \text{ or } t_{1/2} = 0.693V_d/Cl$$

Several physiological events can occur to change V_d or Cl and can therefore influence the $t_{1/2}$ of the drug in the body. For example, if renal function is impaired, the drug's clearance may be reduced and the $t_{1/2}$ prolonged by several hours or several days, in turn prolonging the withdrawal time of the drug. If the animal's fluid balance changes, the V_d may change accordingly. Factors such as the age, nutritional status, percentage of body fat, species, presence of other drugs, and extent of protein binding can all have a significant role in determining the V_d and Cl and hence the $t_{1/2}$ of any drug introduced into the body. For more information on the pharmacokinetics, readers are encouraged to consult Chapters 2 and 3.

Recent work has used physiologically based pharmacokinetic modeling (similar to Figure 3.18) to develop models that incorporate tissue compartment measurements into their structure (Craigmill, 2003; Buur et al., 2005, 2006; Leavens et al., 2014; Lin et al., 2016). These models also allow direct incorporation of statistical estimates of population variability in all processes impacting on residue depletion to be included in the modeling. Their downside is that significant data are required for their solution.

The pharmacokinetic behavior and efficacy of the drug or chemical used to treat a disease process are of major concern early on in the successful management of herd health in food animals. For residue control in food-animal species, the primary purpose of knowing the pharmacokinetic behavior of the drug (i.e., the terminal elimination half-life) is to determine the withdrawal time to avoid residue accumulation in those tissues consumed by humans. A knowledge of what physiological processes affect the half-life is thus essential to determine when a withdrawal time might need to be modified due to disease-induced prolongation of the withdrawal time.

Theoretically, if a tissue tolerance and the dose of drug were known, pharmacokinetic techniques could be used to calculate an individual withdrawal time. For an oral drug, this requires knowing the fraction of the dose administered which is absorbed into the body (e.g., bioavailability). This amount, divided by the V_d, is the initial concentration of drug in the body (C^0). If one were to assume that the loss of drug from the body were only dependent upon the terminal withdrawal time, then adjusting Equation 3.10 by solving for T as the withdrawal time, and substituting K as 1.44 $t_{1/2}$, the following relationship can be derived as

$$\text{withdrawal time} = 1.44 \ln\left(C^0/\text{tolerance}\right)\left(t_{1/2}\right).$$

Of course, this is an oversimplification. In reality, this equation would work if C^0 were the concentration of drug in the target tissue at the end of drug administration, as this amount is dependent upon the λ_2-phase elimination processes. Complex pharmacokinetic models can be used to calculate this number. Then the above equation would be valid. Officially established withdrawal times must take into account all interanimal variability, and thus statistical processing of these data establishes the withdrawal time for the worst-case scenario.

The above equation is useful to gain a perspective on what the withdrawal time is relative to the terminal half-life. Assume that for most antibiotics a "therapeutic" concentration is 10 μg/ml and the tissue tolerance is 0.01 ppm (0.01 μg/ml). One also must assume homogeneous distribution of the drug throughout the body. Thus, withdrawal time equals 1.44 [ln (10/0.01)] $t_{1/2}$, or 9.94 $t_{1/2}$. A withdrawal time for this drug would be equal to 10 half-lives. If the drug has a short half-life (e.g., penicillin), the withdrawal time is short. However, if the drug has a prolonged tissue half-life (e.g., an aminoglycoside), the withdrawal time could exceed a year in the target tissue. Similarly, a drug with a very low tissue tolerance has a longer withdrawal time because ln (C^0/tolerance) is now greater. If a drug is metabolized, the metabolite may determine the withdrawal time ("marker residue") since its half-life is rate limiting.

This "rule of 10" can be derived by assuming that 10 half-lives are required to eliminate 99.9% of an administered dose, as discussed earlier in this section. An examination of half-lives and withdrawal times for a number of approved pharmaceutics confirms this relation as being very conservative relative to human food safety end points (Gehring et al., 2004). In fact, these so-called "half-life multipliers" of 4–5 were often sufficient since the target tolerance for drugs with minimal adverse effects was not at very low concentrations reflective of complete drug elimination. Using a divisor of 5 could actually be considered even more conservative since the additional time added to the withdrawal time would be longer.

This relationship is important to consider when one administers an increased dose of drug. If the dose is doubled, withdrawal time should just be increased by a single half-life. In the above example, the calculation would 1.44 ln (20/0.01), or 10.94 half-lives, which confirms this concept. However, if a disease process changed the half-life by either increasing volume of distribution or decreasing clearance (e.g., kidney disease) causing the half-life to double, withdrawal time should be doubled. This phenomenon supports the observation that *seriously ill animals with altered pharmacokinetics deserve increased attention to ensure complete drug withdrawal.* It is also important to note that withdrawal time is determined in healthy animals, and thus serious disease conditions may cause residues even when the approved dose and official withdrawal time are used. *It is critical for a veterinarian to have a conceptual understanding of this relationship between withdrawal time and half-life before doses are modified in food-producing animals.* When such a withdrawal time is calculated, the animal should be checked with the appropriate rapid screening test.

A clear example of this is seen with the antiinflammatory drug flunixin where prolonged withdrawal times are needed for dosing if severe inflammation is present (Wu et al., 2013; Leavens et al., 2014; Kissel et al., 2015; Smith et al., 2015). The cause of this seems to be both reduced clearance and altered metabolic profile seen with severe inflammatory diseases such as clinical mastitis or modeled using endotoxin injection. The withdrawal time is established in healthy animals yet the drug is labeled to be used in diseased animals. Similar changes in a drug's metabolism would also be expected for drugs such as sulfamethazine, enrofloxacin, and ceftiofur. This change in clearance and metabolic pattern also changes the marker residue ratio and its relationship to total drug, which brings into question the true validity of using this fixed ratio to determine regulatory tolerances (Lin et al., 2016).

For lactating dairy cows and goats, the identical principles apply to determining the milk discard times. The milk discard or withholding time is the time after drug administration when the milk cannot be used for human consumption. This is determined by administering the drug and collecting and analyzing milk until drug concentrations are below the milk tolerance established for that drug. Like withdrawal times, the discard time is product and species dependent. In this case, it is based on the half-life of the drug in the milk. If one knows the concentration of the drug in the milk at the end of drug administration, the milk half-life, and the milk tolerance, the above equation can be used to precisely calculate withdrawal. This has been particularly useful when valuable dairy cows have been accidentally exposed to pesticides (e.g., heptachlor) and pharmacokinetic data were used to determine milk discard times since approved withdrawal times do not exist. In this case, the relevant parameter is the half-life of drug in milk. All of the principles discussed above relative to meat withdrawal apply. The half-life in milk is a function of how the drug is excreted into the milk after systemic administration (or oral or interuterine) or is retained in the udder after intramammary infusion. For example, basic drugs such as erythromycin have longer discard times than acidic drugs such as penicillin because the former tend to distribute more readily into milk due to the pH partitioning phenomenon. Similarly, lipophilic drugs will tend to have longer milk discard times. Discard times are different for these different routes of administration. One must be sure that a drug used as dry cow therapy is not inadvertently administered to a lactating cow, because the residue concerns are different since most dry cow formulations are long-acting preparations.

Drugs Prohibited from Extralabel Use

Certain drugs are prohibited from extralabel use in food animals under AMDUCA (Table 61.1). This is a constantly changing list and thus up-to-date sources should always be consulted. The FDA may prohibit the extralabel use of an approved new animal or human drug or class of drugs in food animals if FDA determines that the extralabel use of the drug or class of drugs presents a risk to the public health, or an acceptable analytical method needs to be established but such method has not been established. A prohibition may be a general ban on the extralabel use of the drug or class of drugs or may be limited to a specific species, indication, dosage form, route of administration, or combination of factors. Finally, extralabel use is prohibited for drugs added to animal feeds.

Residue Prevention

Although public awareness of the drug residue problem in food is high and several governmental agencies spend large amounts of time attempting to control this problem, residues in animal tissues are still an important concern today. The responsibility for residue control and prevention cannot lie solely within a governmental agency; rather, the responsibility must be shared by the government, producers, veterinarians, teachers and academicians, marketing associations, and other interested parties, who must strive for both healthy and efficiently grown animals as well as a safe food supply. Several approaches can be taken to achieve this goal.

The first step in residue prevention is to make individuals and organizations aware of the problem through education. Education of laypersons can be accomplished through a variety of mechanisms: available open lay and veterinary medical literature, computer databases, consultations with veterinary medical personnel, or the efforts of national organizations. A number of national producer organizations have firm initiatives in place to prevent harmful residues in food-producing animals, including dairy (Adams, 1993), beef (Wilkes, 1993), pork (Lautner, 1993), and veal (Wilson and Dietrich, 1993) organizations. Awareness has also been raised concerning some species of companion animals (Macomber, 1993; Kay, 1993).

Failure to observe the correct withdrawal time of a drug was the leading cause of tissue residue violations in two studies (Van Dresser and Wilckie, 1989; Bevill, 1984). Once the importance of residue control in the US food supply is made clear and the importance of observing the correct legal withdrawal times for the drugs used in food-producing animals is understood, the incidence of tissue residue violations will (theoretically) decrease dramatically. Many have reported on other possible ways to reduce or prevent residues from occurring in food animals (Gehring et al., 2006; Riviere, 1991; Marteniuk et al., 1988; Sundlof, 1989; Kindred and Hubbert, 1993; Mercer, 1990). Rapid screening test technology has advanced significantly and has been used to detect low levels of residue contamination in slaughtered animals in a quick manner. As these tests become more sensitive and more widely used, animals with residue contamination can be isolated and removed before reaching the end consumer, further decreasing the residue problem.

Several rapid screening tests are available. They are powerful tools for the veterinarian using a drug in an extralabel manner or in a seriously diseased animal. Once the veterinarian establishes a putative withdrawal time using the principles discussed above, the animal should be tested with the appropriate rapid screening assay to ensure that the prediction is accurate. If a residue is detected, a prolonged withdrawal time should be recommended until a negative screening value is observed. If this practice is followed, the veterinarian will have done everything possible to ensure a residue-free product.

FARAD is a USDA-supported computerized databank of scientific and regulatory information, established in 1982 and reauthorized by the US Congress in 1998, that can assist the veterinarian, producer, or other individuals in making rational choices in preventing drug and pesticide residues in food animals. It is a collaboration between Kansas State University, North Carolina State University, University of California at Davis, and University of Florida at Gainesville. FARAD contains information on: veterinary drug registration information; current label information; foreign registration and safety data; tolerances for drugs and pesticides for tissues, eggs, and milk; withdrawal information; physicochemical properties; pharmacokinetic and toxicokinetic information; residue test information; bibliographic citations; and other information useful in preventing drug residues in food animals. The database is regularly updated to provide users with the most current information available. FARAD is easily accessed by voice telephone (888-USFARAD), the internet (www.farad.org), and e-mail at three regional access centers (Kansas State University, North Carolina State University, and University of California Davis). By contacting an access center, users can obtain any of the current information listed above. FARAD services are free to clientele. FARAD also publishes periodic updates on extralabel drug withdrawal time estimates and other issues as a regular feature in the *Journal of the American Veterinary Medical Association* (FARAD DIGESTS). The program's website (www.farad.org) should be consulted for orientation to its services and current access information. More information on FARAD is available elsewhere (Riviere et al., 1986; Payne et al., 1999, Baynes and Riviere, 2014).

Other sources of drug information pertaining to residues in food animals are also available. The FARAD pharmacokinetic data have been published in book form (Craigmill et al., 2006). The Food and Agriculture Organization (FAO) and World Health Association (WHO) Joint Expert Committee on Food Additives (JECFA) has evaluated several drugs and has published monographs that list toxicity, metabolism, acceptable daily intakes, maximum residue levels permitted for different tissues, and biological fate of drugs in food-producing animals.

The prevention of harmful residues in the edible tissues of our food-producing animals is the responsibility of many producers, veterinarians, professional and layperson associations, and governmental agencies. All of these groups must continue to strive to regulate and utilize the drugs used to prevent or cure animal diseases in a responsible manner in order to prevent the accumulation of harmful amounts of residues in the food supply.

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